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REPRODUCTION IN THE WESTERN MOSQUITOFISH, *GAMBUSIA AFFINIS AFFINIS* (BAIRD & GIRARD), AND ITS USE IN MOSQUITO CONTROL

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REPRODUCTION IN THE WESTERN MOSQUITO FISH, *GAMBUSIA AFFINIS AFFINIS* (BAIRD & GIRARD), AND ITS USE IN MOSQUITO CONTROL

INTRODUCTION

This study of the natural history of the mosquito-fish, *Gambusia*, outside its indigenous area embraces several somewhat diverse yet intimately related biological aspects. The productivity of this poeciliid in ponds, including the factors of age, growth, maturity, reproduction, and survival, is given particular emphasis. The actual productivity in various waters is analyzed with primary emphasis on one pond in central Illinois where conditions for reproduction, survival and growth exceeded all expectations and undoubtedly approached the optimum.

Another phase of the study treats the establishment and acclimation of *Gambusia* into northern Illinois and thence into Michigan.

The data on productivity and on the northward spread are brought to bear on the use of this fish as a mosquito-controlling agent in the northern states. Some data are given on the efficacy of *Gambusia* in the control of these noxious and dangerous insects in Michigan.

The aspects of natural history of *Gambusia* that are given detailed consideration include:

1. The size range and rate of growth of individuals of each sex.
2. The size and age of males and females at first maturity.
3. The length of the reproductive period in male and female mosquitofish.
4. The number of broods to which females give birth during one season.
5. The number of individuals in each of the broods.
6. The length of the period of gestation.
7. The approximate life span of the species in nature.
8. The sex ratio at birth and variations in that ratio during the year particularly as related to age and size.
9. The establishment of the mosquitofish in northern Illinois and thence into Michigan.
10. The conditions required for a population to maintain itself in a climate more severe than that of its natural range.
11. The effectiveness of the mosquitofish as an agent for the control of mosquitoes.

Preliminary collections of *Gambusia* were made from 5 small woods ponds in the Forest Preserve District of Cook County, Illinois, during August 1938. The fish had been placed in those bodies of water

as a mosquito-control measure by the Des Plaines Valley Mosquito Abatement District. Those samples indicated that there were considerable local differences in the various aspects of the life history of this poeciliid and that further collections were desirable. Accordingly, subsequent collections of mosquitofish were made in an attempt to determine the life span of the species in nature and to secure information on its life history and ecology under natural conditions outside its indigenous territory.

During the summer of 1939 mosquitofish were collected at approximately monthly intervals in an attempt to determine the number of broods born during a season, the number of individuals per brood, and any changes that might have occurred in the sex ratio at different times during the summer. The specimens were taken from two ponds, the Argonne Woods Pond and the Sanitary District Lake, in the Chicago region. Both of these ponds had maintained populations of *Gambusia* for at least 3 years and neither had been stocked with additional fish in 1939.

In May 1940, a pond near Mt. Zion, Macon County, Illinois, was stocked with virgin female and male *Gambusia* in the hope that collections later in the summer would yield information concerning the length of the period of gestation, the time necessary for the newborn to reach maturity, the sex ratio in a newly established population, and the total production of fish in the pond. Studies on the length of the gestation period and sex ratio at birth were also carried on in the laboratory.

Mosquitofish were introduced into several waters in southeastern Michigan in June 1941, in an effort to establish the species in that area. Observations were made on the life history, ecology, and the conditions required for a population of *Gambusia* to maintain itself in a climate more severe than that of its natural range. Small samples of fish were taken from time to time for use in the laboratory, but no large collections, similar to those made in Illinois, were taken because of the possibility of seriously depleting the populations as they became established. In addition, many mosquitofish were introduced into natural waters in different parts of the lower peninsula of Michigan as a mosquito-control measure, and this in itself made the killing of large numbers of fish undesirable.

During the summers of 1943 and 1944, experiments on the effectiveness of the mosquitofish as an agent for the control of anopheline mosquitoes were carried on in experimental ponds of the State Fish Hatchery at Drayton Plains, Michigan.

ACKNOWLEDGMENTS

This is a revision of a dissertation accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy at the University of Michigan in 1945.

My gratitude is due the Illinois Natural History Survey, the Forest Preserve District of Cook County, Illinois, the Des Plaines Valley Mosquito Abatement District, and the Institute for Fisheries Research of the Michigan Department of Conservation for the use of many facilities and to various members of the staffs of these organizations for suggestions and advice and for assistance in collecting material. I owe my thanks to Dr. David H. Thompson, of the Forest Preserve District of Cook County, Illinois; Dr. G. W. Bennett of the Illinois Natural History Survey; Professor L. A. Adams of the University of Illinois; Mr. J. Lyell Clarke of the Des Plaines Valley Mosquito Abatement District; Professor G. R. LaRue of the University of Michigan; Dr. Ralph Hile of the U. S. Fish and Wildlife Service; and many others who offered valuable criticisms and suggestions during the course of the work. The Horace H. Rackham School of Graduate Studies of the University of Michigan gave financial aid for the experiments on mosquito control. I am particularly indebted to Professor Carl L. Hubbs of the Scripps Institution of Oceanography for supervising the major portion of the work and for the many invaluable hints in assembling the data and preparing the manuscript.

MATERIAL AND METHODS

Taxonomy of Fish Studied

The kind of *Gambusia* studied during the present investigation is the western mosquitofish, *G. affinis affinis* (Baird & Girard). The genus belongs to the viviparous family Poeciliidae of the order Cyprinodontes. This little topminnow, so called because of its habit of seeking food at the surface is light olive with speckled fins, but is without any conspicuous color markings. The adult females range from 1 to $2\frac{1}{2}$ in total length, whereas the mature males have an average total length of about $1\frac{1}{4}$ inches. As in other poeciliids, mature individuals exhibit sexual dimorphism; the anal fin of the female is small and rounded whereas that of the male is modified into an elongated intromittent organ, the gonopodium. Figure 1 shows an adult male and female *Gambusia a. affinis*.

The genus *Gambusia* was established by Poey (1854), who simultaneously described two Cuban species *Gambusia punctata* and *Gambusia puncticulata*. The generic name was derived from the provincial Cuban word *Gambusino* as indicated by Poey, "El nombre debe su etimología á la palabra provincial cuban *Gambusino*, que significa *nada*, con idea de chasco ó burla: así se dice pescar *Gambusinos* quando no se pesca *nada*." "The name owes its etymology to the provincial cuban word *Gambusino*, which denotes *nothing*, in the jocular or scoffing sense: so it is said to fish *Gambusinos* is to catch *nothing*." The

logotype of the genus, *G. punctata*, was selected by Jordan & Gilbert (1883).

The species with which this paper is concerned was described by Baird & Girard (1854) simultaneously as *Heterandria affinis*, from the Medina and Salado rivers in Texas, and as *Heterandria patruelis*, from the hydrographic basin of the Nueces River in the same state. Evermann & Kendall (1894), who first recognized the identity of the two nominal species, *affinis* and *patruelis*, of identical date, selected the former name.

Regan (1913) listed *Gambusia holbrookii* ranging from Virginia to Alabama, *G. patruelis* from Florida to Texas, and *G. affinis* from Florida to Tampico and the lower Mississippi Valley. Gieser (1923) held that all forms of *Gambusia* in the United States, with the exception of the strikingly dissimilar species from Texas that he listed as *G. senilis*, are of the species *G. affinis*. He regarded *G. holbrookii* and *G. patruelis* as being merely varieties or entirely synonymous and without taxonomic standing. He mentioned, however, 3 types of *G. affinis* that he called "Eastern form" found along the Atlantic coastal plain, "Central form" from the central Mississippi Valley region, and, "Western form" of the Southwest.

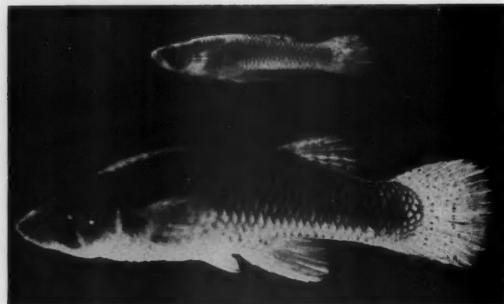


FIG. 1. Adult male (above) and female (below) *Gambusia affinis affinis*.

Hubbs (1926) divided the genus into four subgenera: (1) *Heterophallina*; (2) *Gambusia*, including, under the misidentification of *G. affinis*, the form referred to *G. senilis* by Geiser; (3) *Arthrophallus*, including only the species *G. patruelis*, which ranges from southern Indiana and Illinois to the Gulf of Mexico and along the Gulf Coast from Louisiana to Tampico; and (4) *Schizophallus*, having as the type species *G. holbrookii*, which is very similar to *G. patruelis* and occurs in the Atlantic Coast drainage from New Jersey to Florida, and in the adjacent Gulf Drainage of Florida and Georgia.

Following the introduction of the mosquitofish into Europe, zoologists encountered difficulties in the systematics of the species involved. Information received from Professor Carl L. Hubbs prompted Lindberg (1934) to state that only one species of *Gambusia*, viz. *G. affinis*, had been transported from the United States for use in mosquito control, and that the spe-

cies was divided into two distinct subspecies: *Gambusia affinis affinis* (Baird & Girard) inhabiting the lower Mississippi River drainage from St. Louis to Louisiana on the south, and west along the Gulf Coast to the Rio Grande, and *Gambusia affinis holbrookii* (Günther) ranging from southern New Jersey along the Atlantic Coast to and including Florida and along the Gulf Coast to Alabama. D'Aneona (1939) published a table of characters that he received from Professor Hubbs which pointed out the bases for the taxonomic separation into subspecies. The confusion regarding the application of the name *affinis* had been eliminated by the examination of type material.

The natural range of *Gambusia a. affinis* extends along the Gulf Coast from the mouth of the Rio Grande as far west as Bay St. Louis, Mississippi, and through the Mississippi River and its tributary waters as far north as central Illinois. According to Forbes & Richardson (1920), "This little top-minnow, fairly common in extreme southern Illinois, has been taken by us outside that region only from Quincy, Meredosia, and Pekin." That it is indigenous to that region is indicated by the fact that Jordan & Gilbert (1882) described *Zygonectes inurus* (synonymous with *Gambusia a. affinis*) from the Cache River, Illinois. Luce (1933) reported taking mosquitofish in five collections from the Kalkaskia River between Vandalia and Venedy Station, Illinois. Dr. David H. Thompson, formerly of the Illinois Natural History Survey, in correspondence with the writer, has given the information that *Gambusia* was taken from the Illinois River at seven places between Pekin and the mouth of the river during the summer of 1941. In Indiana, Gerking (1945) found that the distribution of *G. a. affinis* was restricted to the extreme southwestern corner of the state. The first report from much farther north was that of O'Donnell (1935), who listed *Gambusia affinis* from the Fox River basin in northeastern Illinois. As indicated later, it has been determined that this northern occurrence resulted from an introduction. Meek & Hildebrand (1910) did not mention this genus in their "Synoptic List of Fishes Known to Occur within Fifty Miles of Chicago," and Thompson & Hunt (1930) did not collect the species from the waters of Champaign County, Illinois, where the Kalkaskia River has its origin. Hubbs & Lagler (1941) did not include the genus in their "Guide to the Fishes of the Great Lakes and Tributary Waters." From this information the parallel of 40° north latitude may be given as the approximate northern limit of the natural range of *Gambusia*.

Gambusia a. holbrookii ranges from southern New Jersey along the Atlantic coast to the Florida Keys and along the adjacent Gulf Coast as far as Apalachicola, Florida. Between this region and the easternmost fringe of the natural range of *G. a. affinis* these two subspecies intergrade (Hubbs & Walker, MS). *Gambusia a. speciosa* is found in clear water streams tributary to the Rio Grande in Texas, in the Rio

Salado and Rio Juan systems in the Rio Grande basin of northeastern Mexico, and in the upper reaches of the Conchos and Soto la Marina systems of northeastern Mexico (information from Professor Carl L. Hubbs).

Laboratory and Field Procedures

A bright warm day when *Gambusia* literally swarm in the shallow waters of a pond is the most advantageous time for collecting. All collections were made with a one-eighth-inch-square-mesh "Common Sense" minnow seine, 12 feet long and 4 feet deep.

All the fish were killed and fixed in a solution of 10% formalin and were later preserved in 70% alcohol.

Measurements were made to the nearest millimeter from the tip of the snout with the mouth closed to the tip of the caudal fin (total length).

All females from all collections were inspected for young and in each gravid female those embryos which had reached the "eyed stage" and were in a similar stage of development were considered as in the same brood. Actual counts were made of the number of young in the individual broods.

The sex of each fish was determined by an examination of the anal fin. Adult males are readily distinguished by the modification of the anal fin into a copulatory organ, the gonopodium. As Turner (1941: 169) pointed out, the finer structure of the anal fin provides a criterion for the distinction of sex even in very young fish:

"Anchylosis of the joints of the rays begins basally and proceeds apically. The process consists of a gradual narrowing of the segmentation space and finally a complete disappearance of it. At the conclusion there is no trace of a division between segments and the segments are fused end to end. . . . The first visible sex differences in the fin become apparent as a result of the process of solidification. In the male fin the process ceases after the eradication of a single intersegmental space while in the female it continues during the entire life of the animal. . . . The differences between the two sexes are easily detectable and become more pronounced as solidification progresses in the fin of the female and proceeds no further in the fin of the male."

In this study, the anal fins of all fish of a lesser total length than 35 millimeters which did not have a manifest gonopodium, were inspected with the aid of a binocular microscope. Sex ratios are indicated as the numbers of females per 100 males.

During the course of this investigation of the productivity of *Gambusia*, 30,093 mosquitofish (19,150 females and 10,943 males) were measured and sexed. To obtain the average numbers of young per brood carried by females of various lengths at different times of the year and from diverse localities, 2,381 gravid females which yielded 114,311 eyed embryos were examined. The fish were all collected during 1938, 1939, and 1940, from several ponds in Illinois (Table 1).

TABLE 1. Numbers of *Gambusia* collected during 1938, 1939, and 1940, and the numbers of broods counted, from ponds in central and northeastern Illinois.

Pond	Number of fish collected	Number of broods counted
Preliminary collections 1938.....	1,137	272
Argonne Woods Pond 1939.....	9,292	600
Sanitary District Lake 1939.....	12,702	777
Parr's Pond 1940.....	6,962	732
Total.....	30,093	2,381

PRODUCTIVITY

The reproductive efficiency of an animal population is of primary importance in the maintenance of that population. Thus, in order that we may understand the biology of the mosquitofish more fully, and use that fish to its greatest advantage in mosquito control, it is necessary that we have definite information concerning the factors that contribute or detract from its productivity. This is true not only within the indigenous area of the species but also in waters into which it has been introduced.

The factors that effect the reproductive efficiency have been grouped as follows: (1) the rate of growth and the maximum age and size attained by male and female mosquitofish, the size and age at first maturity, the duration of the reproductive period, and the period of gestation; (2) the fecundity of the female as shown by the number and size of the broods born during her lifetime, and the period of senility that may follow reproduction; (3) sex ratio and differential death rate. There are then considered, (4) the data on general populations in various ponds.

AGE, GROWTH AND SIZE

Age and growth studies of a considerable number of freshwater fishes have been reported in the literature. Most of the species so considered grow to a much larger size and are considerably longer-lived than *Gambusia*. As a result those studies do not lend themselves well to comparison with the present investigation. Even the ordinary methods of age and growth determinations are not utilizable because of the brevity of the life span of the mosquitofish. Although annuli are formed on the scales of *Gambusia*, the scale method for age determination is inadequate because in northern and central Illinois this fish seldom, if ever, reaches an age of two years under natural conditions.

It is generally agreed that the maximum age and size of a given species of fish is variable and is determined by the body of water in which it lives, i.e. individuals of a species in one lake may grow to a much larger size or attain a greater age than do fish of the same species in another lake. The differences in length distribution of male and female mos-

quitofish from different waters in the same region are illustrated by data secured from preliminary samples collected August 18-25, 1938, from 5 permanent woods ponds located in the Palos Hills region southwest of Chicago, that ranged from 3 to 6 acres in extent and had maximum depths of 3 to 5 feet. The shorelines were somewhat marshy in all ponds and each supported a heavy growth of vegetation. The length distributions varied widely from pond to pond (Table 2). In each population, however, the females averaged longer and exhibited a greater range of length than the males. All of the fish taken in these 5 collections were young of the year with the exception of 3 individuals. Scale samples from those 3 fish, one female 53 mm. long from Pond 2, and two females, 54 and 57 mm. long respectively from Pond 5, indicated, by the presence of one annulus, that they had been born during the previous summer. None of the scales from male fish in any of the collections bore annuli. The largest female taken in any of the collections was 57 mm. long and the largest male was 34 mm. long. Both were collected from Pond 5.

In order to secure more adequate information on the rate of growth and the maximum size and age of *Gambusia* under natural conditions in northern Illinois, collections were made May 19, June 9, July 13, August 14, September 13, and October 19, 1939, in 2 ponds that were markedly different in type of habitat and natural fertility. The Argonne Woods Pond lies northeast of the intersection of 95th Street and 100th Avenue in Palos Township one mile south and one and a half mile east of Willow Springs Village. This permanent marsh pool, 5 to 6 acres in area, has a more or less regular saucer-shaped basin with a maximum depth of 5 feet. A levee which raised the water to its present level was built along the east shore during the winter of 1937-38. A dense oak-maple woods completely surrounds the pond and approaches the shoreline in many places. A heavy growth of aquatic vegetation, consisting primarily of *Ceratophyllum demersum*, *Myriophyllum* sp., *Potamogeton* sp., and *Lemna* sp., was present and there were many cattails (*Typha* sp.) at various points along the shore. This pond was considered to be high in productivity. The Sanitary District Lake, formed by a depression in the ground at the time the Sanitary and Ship Canal was built, is about 10 acres in extent and is located about half a mile west of Lawndale Avenue between the Des Plaines River and the Sanitary and Ship Canal in the village of Summit. The surrounding terrain is largely clay and limestone and the pond was not considered to be very productive. Various prairie grasses and a few cottonwood trees were the only vegetation in the immediate vicinity of the pond. No rooted aquatic plants were present. A levee constructed at the west end of the lake 10-15 years ago raised the water to its present level. The maximum depth of the lake is about 8 feet near the levee and there is a broad expanse of shallow water at the east end.

Data on age and growth of mosquitofish in a newly

TABLE 2. Number of male and female mosquitofish in collections from each of five ponds in Cook County, Illinois, August 18-25, 1938.

Total length (millimeters)	POND 1		POND 2		POND 3		POND 4		POND 5	
	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀
14.....	1
15.....	1
16.....	..	1	..	1	2
17.....	7	6	..	1
18.....	1	5	..	24	7	1	1
19.....	7	9	5	26	10	2
20.....	4	20	6	22	8	1	..
21.....	7	26	14	64	14	3	1
22.....	2	12	16	63	12	2	2	1
23.....	5	15	10	52	6	4	10	1	..	1
24.....	1	4	10	14	8	2	5	1
25.....	1	5	13	38	13	1	13	..	2	..
26.....	2	2	7	12	20	2	20	1	1	2
27.....	3	6	10	21	2	20	1	1	1	2
28.....	1	10	3	5	2	9	1	3
29.....	1	5	6	5	..	4	5	5
30.....	5	1	2	11	1	2	5	..
31.....	5	..	1	13	3	1	2	..
32.....	5	5	2	2
33.....	8	..	4	1	2	..
34.....	21	..	7	1	3	..
35.....	28	..	8	..	1
36.....	21	..	4	3	..
37.....	7	..	3	..	1	..	1	..
38.....	2	..	1	..	7	..	5	..
39.....	2	..	1	1	..
40.....	7	..	1	..	1	..	10	..
41.....	10	..	1	6
42.....	7	2	4
43.....	1	3
44.....	1	4
45.....	2
46.....	1	3
47.....	3
48.....	2
49.....
50.....
51.....
52.....
53.....	1
54.....	1
55.....
56.....
57.....	1
Total.....	30	104	102	473	136	62	111	29	12	78
Average length.....	21.3	21.6	23.8	25.8	23.5	31.3	26.9	33.4	27.8	36.2
Percentage of collection.....	22.4	77.6	17.8	82.2	68.7	31.3	79.3	20.7	13.3	86.7

established population were secured from collections from Parr's Pond during 1940. This pond, located near Mt. Zion, Macon County, was formed by constructing a dam across a shallow ravine in a pasture to provide a watering place for livestock. At the time it was stocked with fish in May, 1940, the pond was roughly triangular in shape, 75 feet long and 35 feet wide, with a maximum depth of 2 feet. During the following summer the water level fluctuated according to the amount of rainfall, and by October of the same year the size of the pond had shrunk to 50 feet in length and 20 feet in width with a maximum depth of 12 inches. There was no submerged aquatic vegetation. Probably due to the continuous

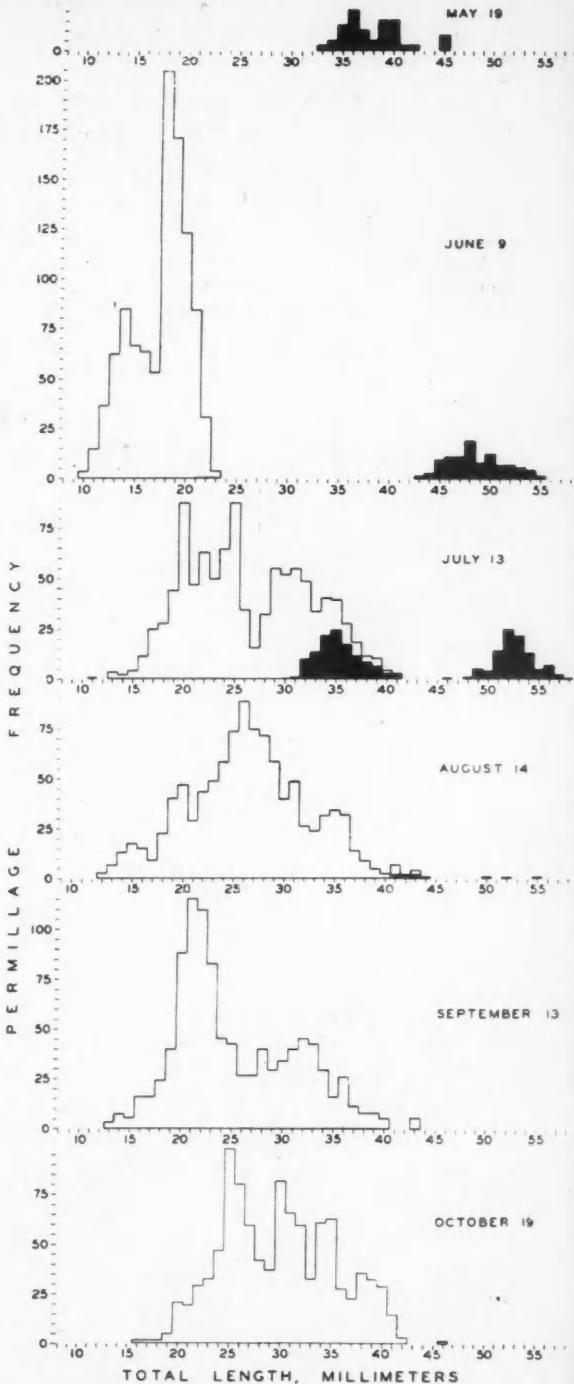


FIG. 2. Length frequencies of all female mosquitofish taken in each of six collections from the Argonne Woods Pond, Cook County, Illinois, during 1939. The shaded areas indicate the gravid females, the unshaded the non-gravid.

deposition of excreta by the cattle and hogs pastured in the area, plankton flourished throughout the summer. The surrounding terrain was gently sloping grassland with a high clay bank on the north side and a few small willows grew along the shore.

LENGTH DISTRIBUTION AND RATE OF GROWTH OF FEMALES

Respective totals of 6,231 and 8,573 female mosquitofish were taken in 6 collections each from the Argonne Woods Pond and the Sanitary District Lake and 3,600 females were secured in 4 collections from Parr's Pond. Those fish that can safely be assumed

to have been present in the ponds prior to the liberation of the first brood of offspring will be referred to as the "parent" stocks and any fish born subsequent to that date will be considered as "offspring." Parent fish were present in the first 4 but were not taken in the last 2 collections from the Argonne Woods Pond, whereas such fish were present in all 6 of the collections from the Sanitary District Lake. In Parr's Pond only one fish of the original planting was found in the first collection and none were present in the subsequent collections. Offspring were taken in the last 5 collections from the Argonne Woods

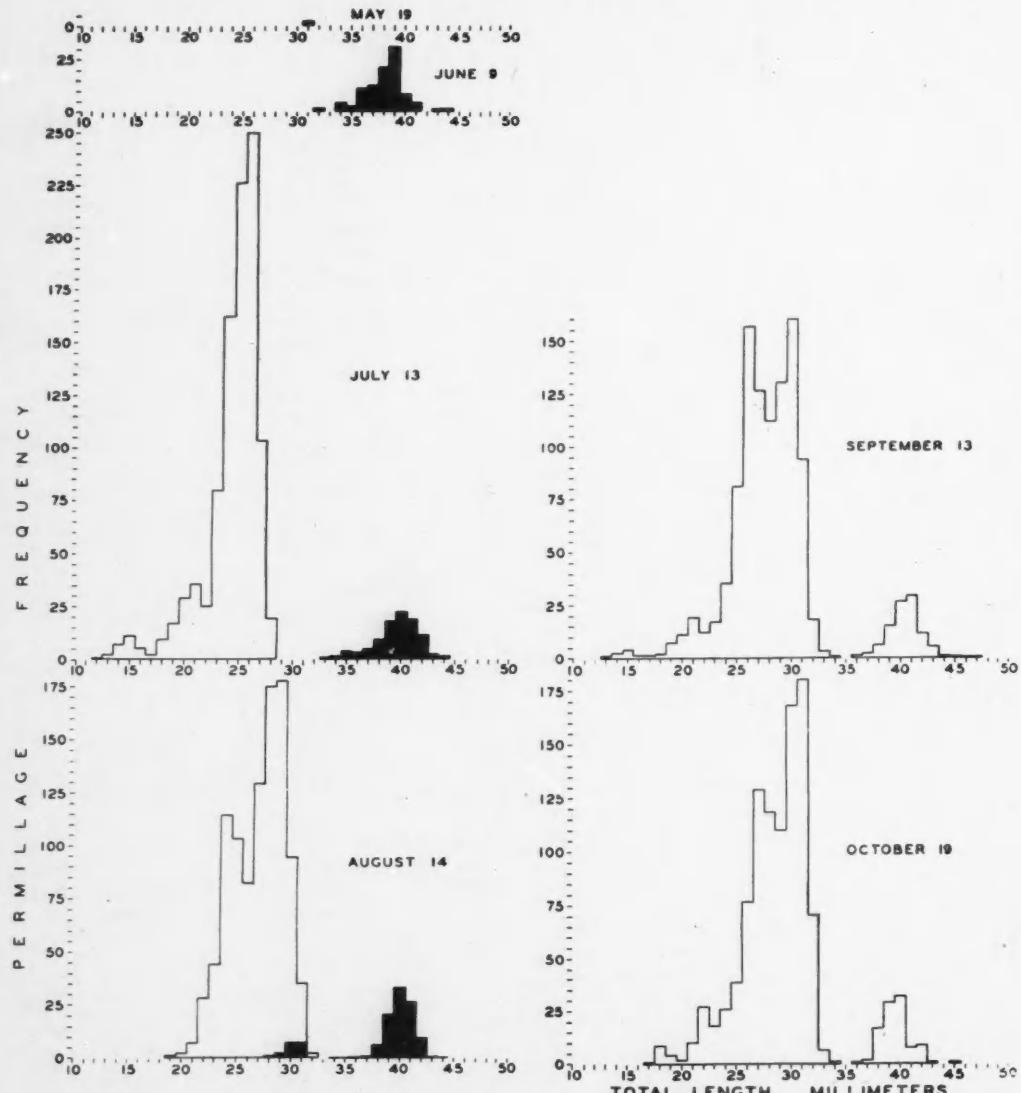


FIG. 3. Length frequencies of all female mosquitofish taken in each of six collections from the Sanitary District Lake, Cook County, Illinois, during 1939. The shaded areas indicate the gravid females, the unshaded the non-gravid.

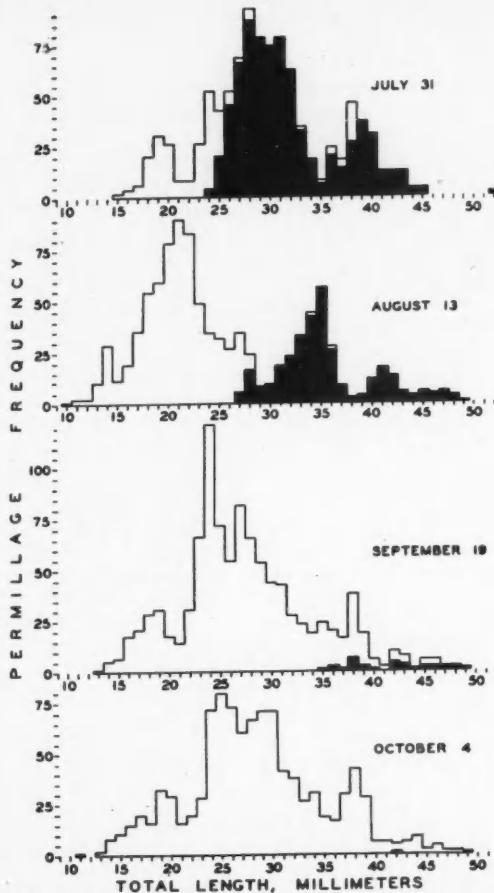


FIG. 4. Length frequencies of all mosquito fish taken in each of four collections from Parr's Pond, Macon County, Illinois, during 1940. The shaded areas indicate the gravid females, the unshaded the non-gravid.

Pond, in the last 4 samples from the Sanitary District Lake, and in all collections from Parr's Pond.

The numbers and size ranges of all females in each collection are given in Table 3 for the Argonne Woods Pond, Table 4 for the Sanitary District Lake, and Table 5 for Parr's Pond. The length frequencies are graphically represented in Figures 2, 3, and 4 respectively.

AGE AND GROWTH OF PARENT FISH

The parent stock of *Gambusia* in the Argonne Woods Pond grew more rapidly and to a larger size than did those in the Sanitary District Lake. The average size of the parent fish in the Argonne Woods Pond increased from 37.9 mm. on May 19 to 52.6 mm. on July 13, a growth of 14.7 mm. in a period of 55 days. The parent fish in the Sanitary District Lake grew much more slowly; from an average length of 38.1 mm. on June 9 to one of 40.4 mm. on September 14, an increase of only 2.3 mm. in 96 days. In the

Argonne Woods Pond, the females reached a maximum length of 59 mm. (Table 3) by the middle of July in their second summer of life, after which time they began to die off rapidly (only a few attained a greater length than 56 mm.). The parent females in the Sanitary District Lake grew to a maximum length of 47 mm. (Table 4) but only relatively few attained a greater length than 43 mm. The numbers of these fish in the collections did not indicate any great mortality until after September 13 when they became scarce and also appear to have stopped growing. Fair numbers of the parent stock lived through their second summer in the Sanitary District Lake, however, as they were present in the October collection. The presence of so many parent fish in October raises the interesting possibility of survival into the third summer. However, none of the fish taken early in the season from either of the ponds bore more than 1 annulus on their scales. As mentioned earlier, only one of the original fish stocked in Parr's Pond was taken in the collections. That fish was known to have been in its second summer of life (1 annulus). Collections made in Parr's Pond by Dr. George W. Bennett of the Illinois Natural History Survey the following spring (1941) failed to reveal any of the original stock.

Apparently the fish in the Argonne Woods Pond and Parr's Pond, having grown rather rapidly and

TABLE 3. Numbers of female mosquito fish, both parent and offspring, with minimum, average, and maximum total lengths in millimeters, in each of six collections from the Argonne Woods Pond, Cook County, Illinois, 1939.

Date of Collection	OFFSPRING			PARENTS				
	Number	Length			Number	Length		
		Min.	Av.	Max.		Min.	Av.	Max.
May 19.....	39	33	37.9	45
June 9.....	1,321	10	17.4	23	74	43	48.8	59
July 13.....	1,367	11	26.4	41	215	46	52.6	58
August 14.....	2,214	12	26.5	44	4	50	51.8	55
September 13.....	371	14	25.3	43
October 19.....	624	16	29.9	46

TABLE 4. Numbers of female mosquito fish, both parent and offspring, with minimum, average, and maximum total lengths in millimeters, in each of six collections from the Sanitary District Lake, Cook County, Illinois, 1939.

Date of Collection	OFFSPRING			PARENTS				
	Number	Length			Number	Length		
		Min.	Av.	Max.		Min.	Av.	Max.
May 19.....	2	31	31	31
June 9.....	71	32	38.1	44
July 13.....	1,214	12	24.4	28	324	33	39.5	44
August 14.....	811	19	26.9	32	917	34	40.1	44
September 13.....	1,863	13	27.4	34	398	36	40.4	47
October 19.....	2,777	17	28.3	34	196	36	39.6	45

TABLE 5. Numbers of female mosquitofish, both parent and offspring, with minimum, average, and maximum total lengths in millimeters, in each of four collections from Parr's Pond, Macon County, Illinois, 1940. The single parent fish was from the original planting.

Date of Collection	OFFSPRING			PARENTS				
	Number	Length			Number	Length		
		Min.	Av.	Max.		Min.	Av.	Max.
July 31.....	494	15	29.8	45	1	52	52	52
August 13.....	1,042	10	25.6	49
September 19.....	920	13	27.1	49
October 4.....	1,144	11	28.3	49

to a larger maximum size as compared with those of the Sanitary District Lake, disappeared from the population very shortly after the end of the reproductive period, whereas those from the Sanitary District Lake, having grown more slowly and to a smaller maximum size, continued to live on for some time. In both cases, however, the parent fish did not survive to the third summer (2 annuli). The fact that the parent females in all of the ponds disappeared from the populations after their reproductive period regardless of their size and did not survive to their third summer of life leads to the belief that the maximum life span of these individuals seldom, if ever, extends over more than two summers.

In all of the collections of mosquitofish made by the writer, the largest female individual encountered was 63 mm. long. It was taken in her second summer of life from a small artificial pond in Oakland County, Michigan. A female *G. a. affinis* of similar length was the largest specimen in any of the collections of the Museum of Zoology at the University of Michigan.

SIZE AND GROWTH OF OFFSPRING

The newly born young of *Gambusia* are usually 8-9 mm. long. Determination of growth of the offspring from the length-frequency data in Figures 2, 3, and 4 is made somewhat difficult by the presence of several broods of young that were born at different times during the summer. These broods of young, cast at rather regular intervals, reflect their growth to a certain extent by modes in their length frequencies. These modes, however, may be obscured in samples taken late in the summer because of variations in the periodicity at which broods are liberated and because individuals of the first brood of offspring, having become mature, give birth to offspring at intervals not necessarily coincident with those of the parent stock.

The first offspring in the Argonne Woods Pond were present in the collection of June 9. There was a rapid increase in the length of these individuals early in the season followed by a slower growth rate later on. From a length of 8-9 mm. at birth (about June 1), they exhibited a maximum increase in length of 14 mm. the first 9 days and within 43 days after

birth had attained a maximum total length of 41 mm. However, in an additional 32 days (75 days after birth) the maximum size was increased to only 44 mm. Apparently this brood had begun to die off by the middle of August inasmuch as it was not manifest in the September or October collections. The actual rate of growth of the other broods is obscure.

No offspring were taken in the collections from the Sanitary District Lake until July 13. The first brood, probably born shortly after the middle of June (based on the stage of development of embryos carried by females in the June collection), did not grow as rapidly or attain so large a size as those in the Argonne Woods Pond. These more slowly growing individuals reached a maximum size of 28 mm. in the first 25-30 days of life (an increase in length of 19 mm.) and a month later had grown only an additional 4 mm. If the largest individuals of this first brood were still present at the time of the October collection, the maximum size could not have been greater than 34 mm.

The offspring in the population of Parr's Pond grew even more rapidly and to a greater length than those in the Argonne Woods Pond. The first brood was born about July 8 (based on an estimate of 49 days, from the time of stocking, for incubation and attainment of maturity—see page 15) and had attained a maximum length of 45 mm. by July 31—an increase of 36 mm. in 23 days. In the subsequent 13 days those fish grew an additional 4 mm. to a maximum size of 49 mm. Similarly as the fast-growing fish in the Argonne Woods Pond the individuals of the first brood in Parr's Pond began to disappear from the population by mid-August and were not present in the September and October collections.

Thus, individuals of the first brood of offspring in rapidly growing populations of *G. a. affinis* (Argonne Woods Pond, Parr's Pond) live only a few months and seldom, if ever, survive to the second summer of life, whereas, those of a more slowly growing population (Sanitary District Lake) tend to live longer and may survive to the second summer. In both the fast- and slow-growing populations, however, individuals of the broods subsequent to the first, although they grow just as rapidly and attain as large a size as those of the first brood, apparently survive the winter and are present in the population of the following year as the parent stock.

SIZE AND AGE AT FIRST MATURITY AND LENGTH OF THE REPRODUCTIVE PERIOD IN FEMALES

In a fish that is as promiscuous in its breeding habits as *Gambusia* there is little chance that copulation will not take place as soon as the female is physiologically ready to bear young. Hence the presence of fertilized ova is the best available macroscopic indication for the attainment of maturity in female mosquitofish. The age and size at first maturity are dependent on the time of year when the female offspring are born and on the rate of growth. In all

of the ponds observed in this study there was an abundance of mature male mosquitofish.

The numbers of gravid and non-gravid female mosquitofish which had attained a length as great as that of the smallest gravid female taken in the collections from the Argonne Woods Pond, the Sanitary District Lake, and Parr's Pond are given in Tables 6, 7, and 8 respectively. All females taken in the May and June collections from the Argonne Woods Pond and the Sanitary District Lake were gravid and had obviously survived from the preceding summer. No samples were collected from Parr's Pond until July 31. Gravid young-of-the-year females were first taken in the July collections from all 3 ponds.

The smallest gravid mosquitofish was taken from the Argonne Woods Pond on July 13. It was 31 mm. long (Table 6) and was probably not more than 6 weeks old (see page 11). Among the other young of the year in the July collection, the fish that had reached a length of 33 mm. were predominantly gravid and all those 35 mm. or more in length carried eyed embryos. Inasmuch as the smallest gravid female carried eyed embryos about midway in their development, it is apparent that they had reached maturity at an earlier date and probably were considerably smaller when they first became mature. In the August collection from the Argonne Woods Pond only a part of the female offspring whose lengths were 41 mm. or greater were gravid and none of the many individuals between 31 and 41 mm. long carried young. Also one of the parent fish (50 mm. long) taken in that collection was not gravid. No gravid fish were collected in September or October.

In the Sanitary District Lake (Table 7), the smallest gravid female was 26 mm. long. It was collected on July 13. Along with 8 others it carried embryos that had not yet reached the eyed stage. None of the young of the year taken in this lake on July 13 carried eyed embryos. These young, probably 4-5 weeks old, were either missed in the collection of June 9 or were born very shortly thereafter. There was no sharp demarcation between the lengths of the gravid and non-gravid females in the July or August collections.

The smallest gravid females in the collections from Parr's Pond were 24 mm. long (Table 8). In all probability they were members of the second brood of offspring liberated by individuals of the original stock (see page 12) and were not more than 4 weeks old. In the July collection the females that were 26 mm. or more in length were predominantly gravid and all but 21 of the 276 females 29 mm. long or longer were gravid. In the August collection those that had reached a length of 28 mm. or more were predominantly gravid and only 8 of the 316 individuals 30 mm. or more in length did not carry young. In that sample all females that were 39 mm. long or longer carried young. In Parr's Pond, but in none of the other Illinois ponds, a few of the females taken during September and October were gravid.

TABLE 6. Numbers of gravid and non-gravid female mosquitofish which had attained the length of the smallest gravid female (31 mm.) taken in six collections from the Argonne Woods Pond, Cook County, Illinois, 1939. No non-gravid females were taken in the first two collections and no gravid females were taken in the last two.

Total length (millimeters)	May 19 Gravid	June 9 Gravid	July 13 Gravid	July 13 Non-gravid	August 14 Gravid	August 14 Non-gravid	September 13 Non-gravid	October 19 Non-gravid
31	1	74	..	105	13	41
32	22	44	..	58	17	37
33	1	..	30	16	..	53	16	20
34	2	..	48	7	..	70	11	38
35	5	..	54	76	6	39
36	8	..	38	71	12	17
37	4	..	25	31	2	14
38	2	..	18	20	3	22
39	6	..	14	13	3	23
40	6	..	6	5	2	18
41	1	..	3	..	4	9	..	9
42	1	1	3	..	2
43	..	1	4	5	2	..
44	..	2	1	1
45	3	7
46	..	8	1	1
47	..	8
48	..	14	2
49	..	6	12
50	..	9	10	..	1	1
51	..	5	30
52	..	5	54	..	1
53	..	4	46
54	..	3	28
55	..	1	11	..	1
56	15
57	5
58	1
59	..	1
Total	39	74	474	141	13	521	87	278

In the collections from the Argonne Woods Pond and the Sanitary District Lake single modes in the length-frequency distributions indicate that only the first brood of offspring reached maturity during the first summer of life. In comparable data from Parr's Pond, however, there are 2 apparent modes in the length frequencies of each of the first 3 collections, indicating that not only the first brood, but the second as well, reproduced during the first summer. In all ponds the first offspring were born before July 13 and their age at first maturity was 4-5 weeks. Further, these broods, as reflected by their numbers in succeeding collections, were rapidly disappearing from the population by late summer. Thus it may be assumed that, in Illinois, mosquitofish which become gravid during their first summer of life do not survive to the following summer and the length of their reproductive period is limited to 8 or 10 weeks.

On the other hand, non-gravid offspring as large or larger than the gravid individuals were present in the populations later in the year. It is not known why the female offspring taken in August (second brood in the Argonne Woods Pond and Sanitary District Lake; third brood in Parr's Pond) were not gravid, although they were as old and as large as

TABLE 7. Numbers of gravid and non-gravid female mosquitofish which had attained the length of the smallest gravid female (26 mm.) taken in six collections from the Sanitary District Lake, Cook County, Illinois, 1939. No non-gravid females were taken in the first two collections and no gravid females were taken in the last two.

Total length (millimeters)	May 19 Gravid	June 9 Gravid	July 13 Gravid	July 13 Non-gravid	August 14 Gravid	August 14 Non-gravid	September 13 Non-gravid	October 19 Non-gravid
26			1	314	67	292	213	
27			6	120	105	236	359	
28			2	22	1	141	209	329
29					2	142	243	305
30					6	71	298	467
31	2				6	23	175	499
32		1				2	34	195
33				4		7	19	
34		3		5	1		1	3
35			2	11	1			
36			8	10	2	3	5	2
37			9	6	10		8	4
38			15	19	12	11	46	25
39			22	30	20	57	134	60
40			6	66	7	145	163	107
41			3	54	8	156	88	118
42				38		68	21	47
43			1	7	1	9		22
44			1	5		3	2	
45							1	1
46							1	
47							1	
Total	2	71	245	544	468	1,025	1,893	2,585

the gravid young-of-the-year females collected in July. Larger offspring (earlier broods) collected at the same time were gravid. If ample time remained for those older offspring to bear a brood of young certainly time must have been sufficient to allow the younger ones to follow suit. Perhaps the younger ones, although of a size and age comparable to the older individuals when they bore their first brood, had not become physiologically ready to bear young. The fact remains that individuals of the later broods (born after mid-July) did not become gravid but rather survived the winter and were present at the beginning of the following season as the parent stock.

As previously stated, the parent fish, when taken in the May collections from the Argonne Woods Pond and Sanitary District Lake, were gravid. These fish, having been born sometime after mid-July of the preceding year, were 8-10 months old when they first became gravid. They continued to bear young until they disappeared from the population during July and August. The numbers of these fish, as reflected by the length-frequency distributions in the first 4 collections, had disappeared by September. Having been gravid since early May and, in some cases, continuing to bear young until late August these individuals exhibited a reproductive period of about 14-15 weeks.

Sokolov (1936) recorded an average growth among adults of *Gambusia* from 34 mm. on June 9 to 42 mm.

on August 27, an increase in length of 8 mm. in 79 days. Among the offspring he found that growth proceeded from 7.6 mm. at birth to 23.1 mm. at an age of 37 days, an increase of 15.5 mm. Further, Sokolov maintained that the offspring reached puberty within 36-40 days after birth but that the peak of fecundity was not reached until the fish attained a length of 45-50 mm., a size that corresponded to an age of 2-3 years as shown by an analysis of their scales. The largest fish attained a length of 50-55 mm. The fish referred to by Sokolov had been introduced into rice fields in Turkestan as a mosquito-control measure. Sella (1929) found that only a small number of mosquitofish attained an age of 2 years when living under "natural conditions" in stock ponds in central Italy. Regarding the question of maximum age among mosquitofish under natural conditions, the writer is in agreement with Sella inasmuch as no fish taken from the Argonne Woods Pond or the Sanitary District Lake, where populations of *Gambusia* had maintained themselves for several years previous to the time of collection, had entered their third year summer of life (2 annuli). Sokolov, in admitting that mosquitofish reached maturity when 36-40 days old and then maintaining that they did not reach their peak fecundity until an age of 2-3

TABLE 8. Numbers of gravid and non-gravid female mosquitofish which had attained the length of the smallest gravid female (24 mm.) taken in four collections from Parr's Pond, Macon County, Illinois, 1940.

Total length (millimeters)	July 31 Gravid	July 31 Non-gravid	August 13 Gravid	August 13 Non-gravid	September 19 Gravid	September 19 Non-gravid	October 4 Gravid	October 4 Non-gravid
24	2	24		36		112		82
25	10	11		33		66		91
26	23	3		28		50		83
27	33	1	6	30		75		69
28	43	3	17	8		60		76
29	39		9	2		49		81
30	37		10			40		81
31	39		19			39		47
32	31		23	1		25		43
33	16	1	34			21		30
34	9		44	2		17		35
35	3	1	59		1	21		21
36	10	2	27	2	2	16		19
37	7	2	9		1	14		34
38	13	10	3	3	6	29		48
39	15	3	4		2	15		32
40	13	2	12		1	4		7
41	6		18					7
42	6		14		4	5	1	5
43	6		6		3	4		7
44	2		4		1	1		10
45	2		6		2	3		3
46			5		2	3		5
47			6			2		2
48			4			2		2
49			1			1		1
50								
51								
52	1							
Total	366	63	340	145	25	674	1	921

years, inferred that the rate of growth between those ages was extremely slow. After having reached a length of 23 mm. in 37 days it required an additional 2 years for them to double their length and reach the height of the reproductive period. The fish in the Sanitary District Lake, which grew almost as slowly as those referred to by Sokolov, reached their peak fecundity at a smaller size (about 40 mm.) but apparently did not survive until their third summer. It may be that the individuals in the rice fields of Turkestan, having grown more slowly, lived to a greater age than those in any of the Illinois ponds.

Zaneo (1933) reported having kept an individual of *G. holbrookii* living under aquarium conditions to an age of at least 5 years. The writer has kept individual mosquitofish in aquaria for nearly 4 years. Such longevity is probably to be attributed to the fact that the life span of many animals may be considerably prolonged when kept under conditions of semi-starvation.

GESTATION PERIOD

Information on the period of gestation was obtained by observing *Gambusia* in aquaria. Four adult female mosquitofish were placed in aquaria with mature males and as soon as copulation had taken place the females were separated from the males and placed in individual aquaria. These fish were fed daily and the aquaria were kept at room temperature. The incubation period ranged from 21 to 28 days (Table 9) and the average of the 14 gestation periods was 23.9 days. It should be mentioned here that some of the broods contained young that were slightly premature, that is, the yolk sac was not completely absorbed.

That the period of gestation under natural conditions is approximately the same as in the aquarium experiment is indicated by observations made in Parr's Pond during the summer of 1940. The patent vent and the still slightly distended body cavity indicated that some of the young-of-the-year female mosquitofish taken on July 31 had released a brood of young very recently. Here, the third generation made its appearance within 71 days after the pond was stocked. During that time the virgin females originally placed in the pond were fertilized, liberated a brood of young, which in turn reached maturity, were fertilized, and gave birth to a brood. Thus, the 71-day

period is to be divided into 2 periods of gestation and the period required for the first brood to reach maturity. Using a figure near the minimum observed period of gestation and assuming that the duration of both incubation periods was the same and that the time required for the young to reach maturity was slightly under 4 weeks, a division of the 71-day interval into 2 gestation periods of 22 days each and a lapse of 27 days for the young to reach maturity seems a reasonable approximation. It has been shown previously that mosquitofish usually grow much faster under such favorable natural conditions as those in Parr's Pond than they do in aquaria.

Seale (1917) recorded periods of gestation for 5 separate broods of young liberated by a single mosquitofish between December 8, 1915 and June 23, 1916. The duration of those periods ranged from 21 to 79 days, with an average of 39.6 days. The longest incubation period is far out of line with the other 4, which averaged 29.8 days. The shortest period of gestation was the same as that observed in the present study but that of the longest was more than 3 times as great.

In observing the parturitions of 29 female mosquitofish living under conditions simulating those of nature, Dulzetto (1928, 1934, 1935) found that the 49 intervals between broods ranged from 19 to 61 days with an average of 27.7 days. The longest period, which occurred between the first and second broods liberated by a female during the summer of 1933, was referred to as abnormal.

Working with *G. a. holbrookii* in the region of Moscow, Samokhvalova (1941), found that, whereas the size of the aquaria, the temperature of the water, and the number of fish per unit of volume of water have an effect on the growth of that poeciliid as well as all other fish, those factors were not decisive in causing the under-development of aquarium-reared *Gambusia*. The decisive factor was held to be direct sunlight. When the aquaria were placed in the direct sunlight, the mosquitofish grew well and began to spawn. When moved back to ordinary laboratory conditions, the fish in the aquaria continued to grow for a short period of time and then apparently relapsed to subnormal growth and reproduction. It was concluded that sunlight is one of the chief prerequisites for the normal growth and spawning capac-

TABLE 9. The date of fertilization, date of birth of each brood, and number of days in the periods of gestation for each brood liberated by four female mosquitofish in laboratory aquaria, 1940.

Female number	Date of fertilization	FIRST BROOD		SECOND BROOD		THIRD BROOD		FOURTH BROOD	
		Date of birth	Days of incubation						
1.....	Aug. 2	Aug. 27	25	Sep. 20	24	Oct. 11	21
2.....	Aug. 2	Aug. 25	23	Sep. 16	22	Oct. 10	24	Nov. 1	22
3.....	Aug. 2	Aug. 27	25	Sep. 19	23	Oct. 12	23	Nov. 2	21
4.....	Aug. 2	Aug. 30	28	Sep. 25	26	Oct. 22	27
Average.....			25.2		23.8		23.8		21.5

ity of mosquitofish under either laboratory or natural conditions.

Gambusia is one of the poeciliids that does not exhibit superfetation (Turner 1937). One brood of young is born before any other ova have matured sufficiently to be fertilized. There is no overlapping of broods. Consequently only a single brood of developing embryos is present in the ovary at any one time and the embryos in that brood are in a similar stage of development. The studies of Kuntz (1913) and especially those of Turner (1937) have adequately outlined the reproductive cycle of *Gambusia*.

The manner in which the young of *Gambusia* are born was observed while collecting data on the duration of the period of gestation. All young were liberated individually. Some were born headfirst, some tailfirst, and some sideways and they were always more or less ball-like when released. Parturition of a single brood required from 23 to 67 minutes. Sometimes a single offspring was released and then, after an interval of several minutes, 3 or 4 were liberated in rapid succession, followed by another time interval and the release of more young. The greatest number of young observed to be released in rapid succession was 6, born in an interval of 3 seconds. The birth of a brood on August 27, 1940, was annotated as follows: ". . . She was seen to give birth to 23 young of which 9 were eaten immediately upon being liberated, the remaining 14 young were removed from the tank. Then the female gave birth to 9 more young in groups as follows: 1, 3, 2, 2, 1, which were devoured as soon as each group was released. Ten other young born later were rescued from the aquarium and placed with those which had been removed previously. . . ." The interval of time required for the birth of the 42 young was 52 minutes. Perhaps the removal of some of the young from the aquarium either lengthened or shortened the process to some degree. As soon as the young were born they sank to the bottom of the aquarium where they remained for several hours, rising to the surface at intervals. Within 5 hours after birth all of the young were actively swimming and feeding.

Ryder (1885) reported the observations of Mr. A. A. Duly on the manner in which *Gambusia* are born:

"The young when born are stated by Mr. Duly to be about three-eighths of an inch in length, and to be expelled in a single mass, consisting of eight to eleven young fishes at a single effort. This mass as soon as it escapes is seen to be composed of infant *Gambusia*, which at once separate and swim away. . . . My informant also tells me that the parent fishes devoured their young as soon as they were born if they were not separated, by transferring one or the other at once to another aquarium. . . . He also noticed that more than one brood seemed to be produced by the same parent consecutively and during the same season. . . ."

The birth of the young as described by Mr. Duly does not conform with observations made during the present study. *Gambusia* were never observed to be born in masses of several individuals but were always

liberated singly though often released in rapid succession. The young at birth, when rounded up, are 2-3 mm. in diameter and it would be physically impossible for any adult female mosquitofish to expel a mass of 8-11 offspring at a single effort. Figure 5 shows a gravid female mosquitofish with her brood of 79 embryos which had been removed prior to birth. These embryos were fully developed and nearly ready to be released. Figure 6 shows an adult male and female mosquitofish together with some newly born young.

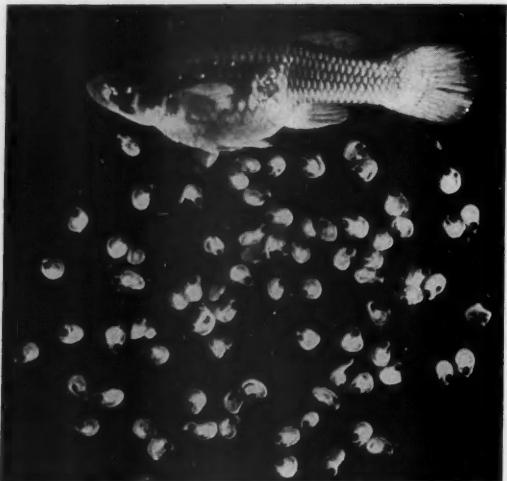


FIG. 5. Gravid female of *Gambusia* and brood of 79 eyed embryos. These embryos are nearly fully developed, the yolk sac having been completely absorbed, and are about ready for birth.

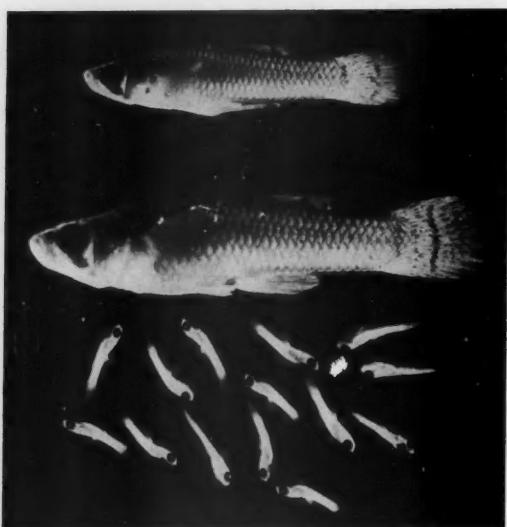


FIG. 6. Adult male and female of *Gambusia* together with young (the female is not the parent). The young are about four hours old.

FECUNDITY

The numbers of ova produced by various species of teleost fishes are roughly proportional, inversely, to the life expectancy, which to a large degree is determined by the extent of parental care. Loss at any immature stage needs to be compensated for by greater fecundity. In some fishes, as the cod and pollock, several million eggs may be shed by a single female, and a number of fresh-water species are known to deposit many thousands of ova at each spawning. Although predation and other factors lead to the destruction of enormous number of eggs, at least enough ova produce young which reach maturity to insure maintenance of the population. *Gambusia* and other Poeciliidae contrast with fishes that produce thousands or millions of unprotected eggs in that they are viviparous and the number of young in a brood rarely exceeds 300. The newborn fish, having been protected from many of the dangers that beset the larvae of oviparous fishes, are at an advanced stage of development and, being very active, begin to fend for themselves immediately. Thus, although the number of individual mosquitofish in a single brood is very small as compared with the number of eggs deposited by most oviparous fishes, nearly all of the ova fertilized in the poeciliids become well-developed young. This viviparity coupled with the release of several broods during each season tends to assure the perpetuation of the race.

The present study shows that the number of young produced by a single female mosquitofish depends on the number of broods liberated during a season, the length of the mother fish, the time of season at which the individual broods are cast, and the locality in which the mother fish lives. These factors are interdependent. The number of young carried by a female of a given size in a fertile pond is larger than the brood carried by a female of the same size in a pond that is not so productive. Similarly, the size of the brood carried by a female of a given length at the height of the reproductive season is larger than that carried by a female of the same length at a later date.

The marked correlation between the brood size and the length of the mother fish and the differences with locality were determined by an analysis of the collections that were made from the various ponds during the summers of 1938-1940. There were wide differences in the sizes of the broods carried by females in the 5 ponds near Chicago in 1938. Furthermore, the data from the Argonne Woods Pond, the Sanitary District Lake, and Parr's Pond showed that the differences were manifest in successive collections taken throughout the summer. The first 2 ponds from which successive collections were made supported standing populations of mosquitofish and were considerably different in type of habitat and natural fertility. Parr's Pond, on the other hand, supported a newly established population and, due to the effects of the continuous deposition of excreta on the surrounding terrain by cattle and hogs pastured in the

area, was probably more fertile than either of the other ponds.

The information on the fecundity of *Gambusia a. affinis* presented here is based on actual counts of 2,380 individual broods that yielded a total of 114,311 eyed embryos. The largest number of embryos in one brood, 315, was carried by a 59 mm. female of the parent stock taken from the Argonne Woods Pond on June 9. The smallest brood carried by female of that stock was one of 10 embryos taken from a specimen 35 mm. long from the Sanitary District Lake. Among the young of the year the largest brood, 218 embryos, was taken from a 47 mm. female from Parr's Pond on August 13, and a single embryo from a 34 mm. fish taken in the Sanitary District Lake on August 14 comprised the smallest brood.

NUMBERS OF BROODS LIBERATED BY A FEMALE DURING A SEASON

It has been previously pointed out (page 12) that the reproductive period in female mosquitofish varies from 8 to 15 weeks, depending primarily on the age at first maturity. Females that mature during the first summer of life usually reproduce over a period of only 8 or 10 weeks, whereas fish that do not reach maturity until their second summer enjoy a reproductive period of 14-15 weeks. Also, it has been shown (page 14) that the average gestation period is about 23-24 days. A fish that reproduces for 8 to 10 weeks liberates 2 or possibly 3 broods, whereas one that continues to reproduce for 14 to 15 weeks gives birth to 4 and possibly 5 broods.

The presence of gravid parent females in the first 4 collections from the Argonne Woods Pond (Table 6) and the Sanitary District Lake (Table 7) indicates that, in each of those populations, the fish which became gravid for the first time during their second summer of life probably bore 4 broods during the season. However, the graphic representations of the length frequencies in Figures 2 and 3 indicate that, in the Argonne Woods Pond, the parent stock liberated at least 4 and possibly 5 broods during the season whereas those in the less productive Sanitary District Lake usually gave birth to 3 broods and never had more than 4. It is evident that the fish in the more productive pond produced a greater number of broods than did those from the Sanitary District Lake. In Parr's Pond (Fig. 4) it appears that the parent stock gave birth to at least 4 broods of young.

Among the offspring in the 3 ponds, the data from the same tables and figures show that only the first brood of young in the Argonne Woods Pond and the Sanitary District Lake reached maturity during the first summer and that in each case they liberated no more than 2 broods during the season. In Parr's Pond, on the other hand, the first 2 broods of offspring reached maturity during their first summer of life and some individuals continued to reproduce until October. Although the data are not conclusive, it seems probable that the first brood, in that newly

established population, liberated at least 3 broods of young and that the second brood liberated 2 and possibly 3 broods.

From the foregoing discussion it appears that, in Illinois, parent mosquitofish liberate an average of 4 broods of young per season and that the offspring, which mature during their first summer of life, give birth to 2 and possibly 3 broods.

RELATIONSHIP BETWEEN THE LENGTH OF THE GRAVID FEMALE AND THE NUMBER OF YOUNG PER BROOD

In general, as was to be expected, the average number of young carried by females of a given size in a single collection was found to increase with the length of the mother fish, in both offspring and parent stocks, in all populations of mosquitofish, throughout the reproductive period. Of the 17 collections taken during the present study (Tables 10-13) the data from 15 of the samples showed increases in the average brood size with the increased length of the mother fish. In each of the other 2 samples the similar lengths of the few gravid females precluded comparison with fish of other sizes. There was some variability in the relationship between the size of the individual mother fish and the number of young when mother fish of similar lengths in the same population

were compared, and also there were some discrepancies in the regular increase of the average brood size of females of increasing lengths. Most of those irregularities, however, were traceable to the small numbers of gravid females of certain sizes in the collections.

At first glance the determining factor in the relationship between the length of the mother fish and the number of young per brood appears to be the physical limitation of size in the gravid female; a small fish does not provide as much space for carrying embryos as does a larger one. If space alone were the determining factor, the number of young would increase approximately in direct proportion to the cube of the length of the mother fish. If that relation held the result would be a regularly increasing number of young per brood as the parent female continued to grow and reproduce. The number of embryos per brood, however, does not increase regularly in that proportion. Furthermore, the rate of increase in the size of the brood per unit length of the gravid female is not constant for all sizes of mosquitofish. Obviously the space provided by the mother is not the sole factor that determines the number of young in a brood.

The data given in Tables 10-13 were rectified by

TABLE 10. Average numbers of eyed embryos taken from female mosquitofish of different lengths in collections from five ponds in the Forest Preserve District of Cook County, Illinois, August 1938.

Total length (millimeters)	POND 1		POND 2		POND 3		POND 4		POND 5	
	Number of gravid females	Average number of eyed embryos	Number of gravid females	Average number of eyed embryos	Number of gravid females	Average number of eyed embryos	Number of gravid females	Average number of eyed embryos	Number of gravid females	Average number of eyed embryos
26	2	21	9	12	1	9	1	15	1	15
27	3	27	9	16	2	26	1	16	1	16
28	1	25	3	15	2	26	1	15	1	15
29	1	35	4	13	1	5	1	16	1	16
30	1	35	3	14	2	32	1	21	1	21
31	1	35	4	17	1	65	3	28	1	14
32	1	35	3	22	5	49	2	36	1	14
33	1	35	8	20	4	62	1	1	1	9
34	1	35	21	24	7	66	1	2	2	9
35	1	35	28	31	7	64	1	50	3	16
36	1	35	21	37	4	79	1	1	1	24
37	1	35	7	38	3	71	1	56	1	25
38	1	35	2	43	1	75	7	56	4	23
39	1	35	2	60	1	126	1	1	3	64
40	1	35	7	58	1	49	1	81	6	54
41	1	35	10	71	1	54	1	68	4	55
42	1	35	7	60	1	122	2	68	3	88
43	1	35	1	49	1	122	1	66	4	87
44	1	35	1	1	1	126	1	1	3	92
45	1	35	1	1	2	122	1	1	2	109
46	1	35	1	1	1	126	1	1	1	174
47	1	35	1	1	1	126	1	1	1	123
48	1	35	1	1	1	126	1	1	1	123
54	1	35	1	1	1	126	1	1	1	123
57	1	35	1	1	1	126	1	1	1	123
Total	7	183	149	4,891	42	2,650	25	1,053	49	2,726
Average	27.1	26.1	34.5	32.8	34.7	63.6	34.7	42.1	41.4	55.6
Percentage of gravid females in total	6.7	...	31.5	...	67.7	...	86.2	...	62.8	...

TABLE 11. Average numbers of eyed embryos taken from female mosquitofish of different lengths in each of four collections from the Argonne Woods Pond, Cook County, Illinois, 1939.

Total length (millimeters)	MAY 19		JUNE 9		JULY 13		AUGUST 14	
	Number of gravid females	Average number of eyed embryos						
31	1	8
32	22	15
33	1	31	30	21
34	2	46	48	24
35	5	50	54	29
36	8	62	38	37
37	4	62	25	43
38	2	64	18	54
39	6	62	14	65
40	6	67	6	86
41	1	73	3	107	4	16
42	1	60	1	9
43	1	172	4	15
44	2	151	1	23
45	3	78	7	166
46	8	201	1	136
47	8	199
48	14	206	2	146
49	6	216	12	150
50	9	207	10	138	1	49
51	5	197	30	136
52	5	232	54	155	1	30
53	4	227	46	156
54	3	238	28	159
55	1	244	11	180	1	49
56	15	184
57	5	191
58	1	99
59	1	315
Offspring:								
Total	259	8,886	10	156
Average length	35.3	..	42.2	..
Average number	34.3	..	15.6
Parent stock:								
Total	39	2,383	74	15,201	215	33,480	3	128
Average length	37.9	..	48.8	..	52.6	..	52.3	..
Average number	..	61.1	..	205.4	..	155.7	..	42.7

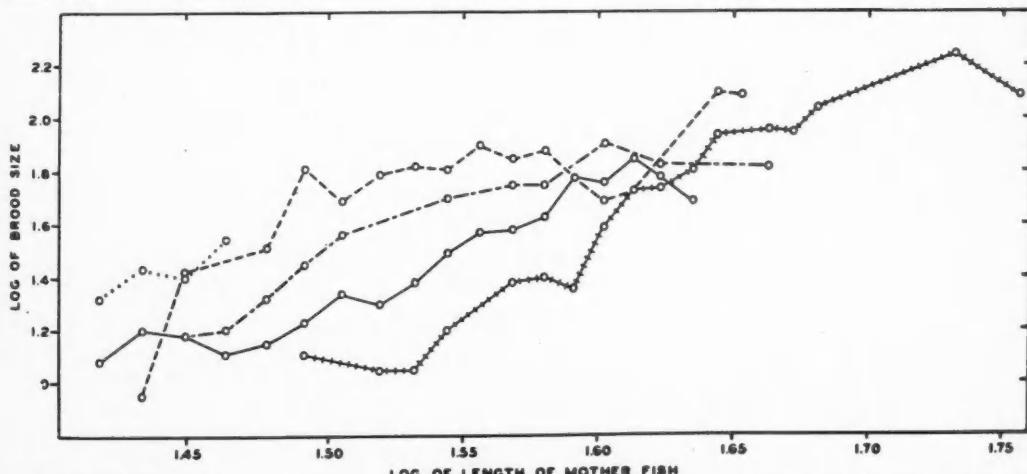


FIG. 7. The rate of increase in the number of young per brood as correlated with the increase in the length of the mother fish in collections from five ponds in the Forest Preserve District of Cook County, Illinois, August 1938. These curves represent the data in Table 10 which have been rectified by plotting the logarithm of the size of the brood against the logarithm of the length of the mother fish. Collections are indicated as follows: Pond 1,; Pond 2, —; Pond 3, - - -; Pond 4, - - - - -; Pond 5, -|-|-|-|-|-|.

TABLE 12. Average numbers of eyed embryos taken from female mosquitofish of different lengths in each of four collections from the Sanitary District Lake, Cook County, Illinois, 1939.

Total length (millimeters)	MAY 19		JUNE 9		JULY 13		AUGUST 14	
	Number of gravid females	Average number of eyed embryos						
28	1	10
29	2	8
30	6	4
31	2	19	6	5
32	1	22
33	3	27	1	1
34	2	22	1	10	1	4
35	8	24	1	19	2	4
36	9	24	6	19
37	15	32	19	20	11	7
38	22	30	39	24	57	7
39	6	37	66	25	145	8
40	3	43	54	28	156	9
41	38	38	68	11
42	1	48	7	52	9	14
43	1	63	5	55	3	16
44
Offspring:								
Total	15	80
Average length	30.1	..
Average number	5.3
Parent Stock:								
Total	2	38	71	2,157	236	6,704	453	3,975
Average length	31	..	38.8	..	40.3	..	40.5	..
Average number	..	19	..	30.4	..	28.4	..	8.8

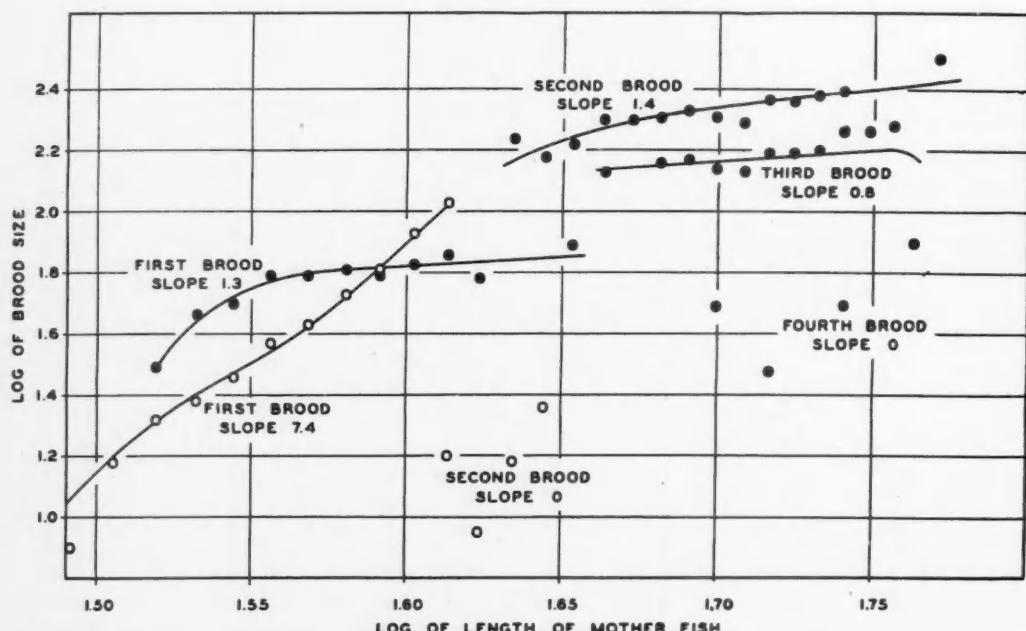


FIG. 8. The rate of increase in the size of the brood as correlated with the increase in the length of the mother fish in successive collections from the Argonne Woods Pond, Cook County, Illinois, during 1939, as given in Table 11. The slope values represent the exponent of the rate of increase as indicated by a straight line drawn through the main part of each curve. The solid dots represent the parent females, the open circles the gravid offspring.

TABLE 13. Average numbers of eyed embryos taken from female mosquitofish of different lengths in each of four collections from Parr's Pond, Macon County, Illinois, 1940.

Total length (millimeters)	JULY 31		AUGUST 13		SEPTEMBER 19		OCTOBER 4	
	Number of gravid females	Average number of eyed embryos						
24	2	9
25	10	18
26	23	18
27	33	23	6	10
28	43	27	17	8
29	39	31	9	12
30	37	36	10	15
31	39	42	19	17
32	31	48	23	20
33	16	52	34	23
34	9	58	44	28
35	3	49	59	31	1	4
36	10	62	27	34	2	10
37	7	81	9	40	1	23
38	13	72	3	63	6	10
39	15	80	4	74	2	9
40	13	83	12	67	1	4
41	6	78	18	67
42	6	95	14	69	4	6	1	31
43	6	103	6	69	3	14
44	2	98	4	71	1	14
45	2	149	6	100	2	56
46	5	95	2	52
47	6	118
48	4	102
49	1	98
52	1*	154
Total	365	16,252	340	12,755	25	428	1	31
Average length	31.6	..	35.4	..	40.5	..	42.0	..
Average number	..	44.5	..	37.5	..	17.1	..	31.0

* From original planting, not included in computation of totals or means.

plotting the logarithm of the average number of embryos per brood against the logarithm of the length of the mother fish (Figs. 7-10). Here the slope of the curve is equivalent to the exponent to which the length of the mother fish is raised in the equation:

$$\text{Number of embryos} = \text{length}^n \text{ of mother fish.}$$

The rectified curves indicate that the greatest rate of increase in the size of the brood as compared with the length of the mother occurred in the first brood produced by the gravid offspring taken in the July 13 collection from the Argonne Woods Pond (Fig. 8). That curve shows that the size of the brood varied as the 7.4 power of the length of the mother fish. Such a rate of increase in a biological relationship is most spectacular and were it to continue, there would result an increase of more than one hundred fold in the number of young with a doubling of the length of the mother. That, of course, would be a physical impossibility. Since the size of the embryos at birth is similar regardless of the size of the mother, the larger fish would have to carry more young than the increase in length would allow and the bellies of such fish would become so distended that they would rupture. Somewhere along the line such a relationship would have to break down as indeed it does. In

the larger fish the fecundity increases at a drastically reduced rate. Even then some of the gravid parent females taken during this investigation were so swollen with young that locomotion was seriously impaired. In those fish the number of young varied only as the 1.4 power of the length of the mother.

For the ponds from which periodic collections were made during the reproductive season, the rectified data indicate that the rate of increase in the relationship between the size of the brood and the length of the mother fish was not the same for the various broods liberated during the season (regardless of the category of the mother fish). Nor was the rate of increase constant within any particular brood produced by females of different lengths within the same category. In most curves the slope was greatest for the second brood born to the mother. Among the parent fish from the Argonne Woods Pond (Fig. 8) the power of increase was approximately 1.3 for the first brood of young, increased slightly to 1.4 for the second brood, and then fell off to 0.8 for the third brood. In the Sanitary District Lake (Fig. 9) the slope of the curve for the first brood of young carried by the parent fish was 3.7, then rose to 5.5 for the second brood, and dropped to 4.2 in the third brood.

In Parr's Pond (Fig. 10), from which there were no adequate data on the fecundity of the parent fish, at least 2 broods of offspring reproduced during their first summer of life. The first brood of young produced by the first mature offspring had been liberated prior to July 31 (see page 15) and it is impossible to say what the relationship between the size of the brood and the length of the mother fish might have been. However, the size of the second brood varied as the 2.3 power of the length of the mother fish and that of the third brood varied as the 1.7 power. Among the individuals of the second brood of mature offspring the size of the first brood increased as the 4.1 power of the mother's length and that of the second brood increased as the 4.8 power. The curves for the fourth brood carried by the first mature offspring and the third released by the second brood of mature offspring in Parr's Pond indicate that for those lots the relationship has broken down completely.

From these data it may be inferred that, with the exception of the gravid offspring in the Argonne Woods Pond, the rate of increase in the size of the brood was generally greatest in the second brood of young liberated by all mother fish regardless of their size or age. Further, the actual numbers of embryos in the second brood carried by mother fish, both parent and offspring, of the various lengths in all ponds were greater than those in any preceding or subsequent broods.

Within a single brood produced by the mother fish of either category the slopes of individual curves changed with the increased length of the female. In the rapidly growing populations of Parr's Pond and

the Argonne Woods Pond, with the single exception of the gravid offspring in the latter pond, the rate of increase gradually diminished as the length of the mother fish became greater. Such a decrease in the rate of increase held in both offspring and parent stocks, except as noted, throughout the summer in both ponds. Among the parent fish from the Argonne Woods Pond the slope of the curve for mother fish 33-37 mm. in length on May 19 was 4.5; for fish 35-39 mm. long it was 2.2; and for the fish above 37 mm. in length it was 1.0. Similarly diminishing slopes in the curves held for subsequent broods carried by the parent stock. Among the offspring in Parr's Pond the slopes of the curve for the first brood of young carried by the second brood of mature offspring gradually decreased from 5.3 for fish 24-28 mm. long, to 4.5 for 26-30 mm. fish, to 4.2 for those individuals longer than 30 mm. Again, similar curves held for data on the young carried by other offspring in that pond.

In the slow-growing fish from the Sanitary District Lake (Fig. 9) the reverse was true; that is, the rate of increase gradually increased with the length of the gravid female. In the first brood of young carried by the parent fish in that population the slope of the curve for the individuals 32-37 mm. long was 0.6. From that figure there was an increase to 3.4 for fish 35-39 mm. long, and to 5.1 for those fish of a greater length than 37 mm. Similar curves were obtained from the data for the 2 subsequent broods.

The reason for such diversity in the relationship between the size of the brood and the length of the mother fish is not readily discernible. Presumably some metabolic factor is involved. Physiological dif-

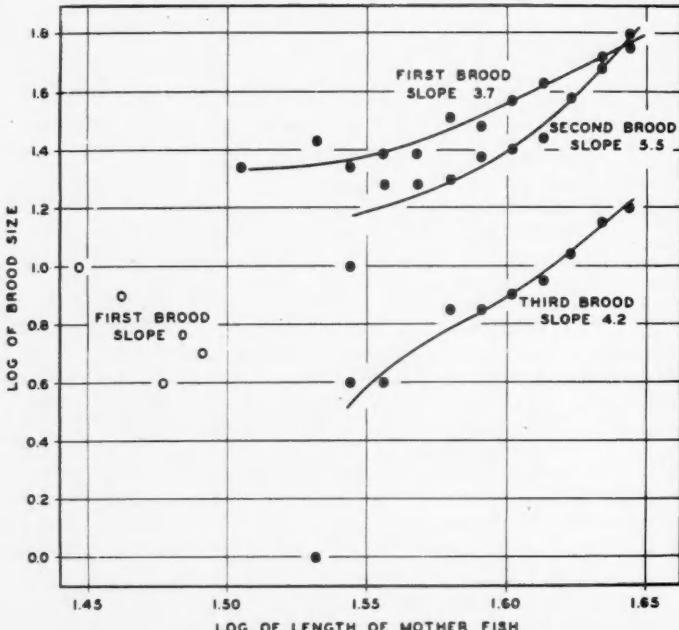


FIG. 9. The rate of increase in the size of the brood as correlated with the increase in the length of the mother fish in successive collections from the Sanitary District Lake, Cook County, Illinois, during 1939, as given in Table 12. The slope values represent the exponent of the rate of increase as indicated by a straight line drawn through the main part of each curve. The solid dots represent the parent females, the open circles the gravid offspring.

ferences, dependent to a certain extent on environmental conditions, may account for the increased or decreased rates of increase. That the space provided by the mother fish is not the sole determining factor for the number of young per brood is evident for several reasons: (1) the size of subsequent broods decreases with increasing size and age of the mother, (2) the approaching end of the reproductive period apparently has some marked effect on the fecundity, and (3) there is considerable difference in the fecundity of fishes of the same size from different localities.

DECREASE IN THE SIZE OF BROODS WITH INCREASING AGE

With the single possible exception of the first brood born to the parent stock, females of the same category in a single population have fewer young per brood as the season progresses. This decrease in fecundity with increasing age holds for both parent fish and offspring (Tables 11-13; Figs. 7-10). The average number of young per brood for parent females of various lengths from the Argonne Woods Pond and the Sanitary District Lake show that even though the actual length of the females increased there was a marked decrease in the size of the broods in successive collections. Similarly, among the mature offspring in Parr's Pond the broods carried by females taken in the second collection were smaller than those taken in the first sample even though the average size of the gravid offspring increased nearly 3 mm. There was a further decrease in brood size as the summer progressed in all cases.

The comparisons given below of the mean numbers of young carried by parent females of similar lengths

on June 9 and July 13 and on July 13 and August 14 for the Argonne Woods Pond and the Sanitary District Lake clearly show those differences:

Mean sizes of broods—Argonne Woods Pond

Date	Females 46-55 mm.		Females 38-44 mm.	
	June 9	July 13	July 13	Aug. 14
Number of females	63	194	179	3
Average size	49.3	52.2	52.5	52.3
Average number of embryos	210.4	152.9	153.3	42.7
Decrease		57.5		110.6

Mean sizes of broods—Sanitary District Lake

Date	Females 35-44 mm.		Females 50-55 mm.	
	June 9	July 13	July 13	Aug. 14
Number of females	67	236	228	449
Average size	38.3	40.3	40.4	40.6
Average number of embryos	30.7	28.4	28.8	8.8
Decrease		2.3		20.0

The young-of-the-year fish in Parr's Pond show agreement with the parent fish in the 2 ponds near Chicago in that the size of the broods decreased as the summer progressed:

Mean sizes of broods—Parr's Pond

Date	Females 27-45 mm.		Females 35-46 mm.	
	July 31	Aug. 14	Aug. 14	Sept. 19
Average number of females	330	324	167	25
Average size	32.2	34.9	38.2	40.5
Average number of embryos	47.4	34.3	49.9	17.0
Decrease		13.1		32.9

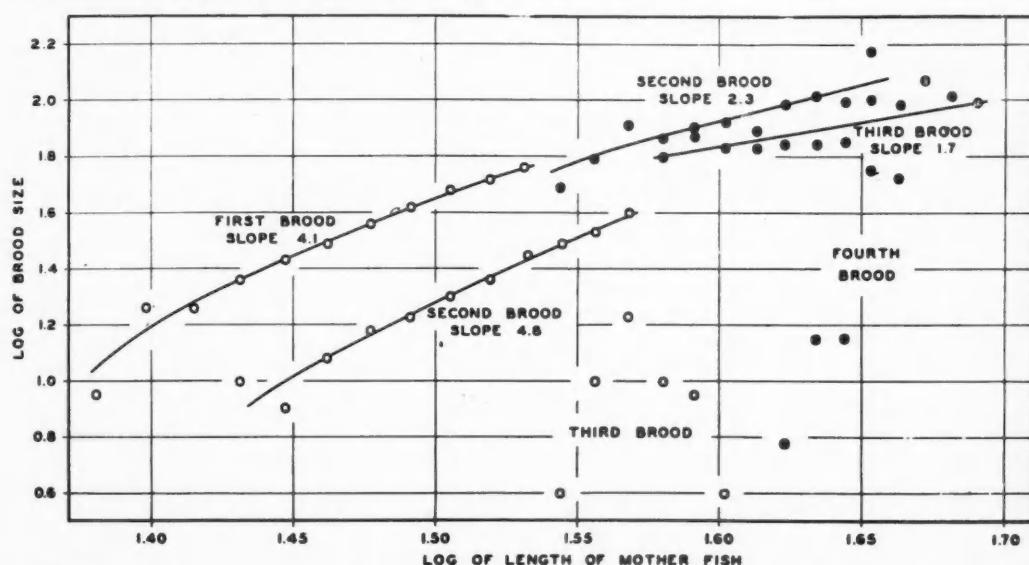


FIG. 10. The rate of increase in the size of the brood as correlated with the increase in the length of the mother fish in successive collections from Parr's Pond, Macon County, Illinois, during 1940, as given in Table 13. The slope values represent the exponents of the rate of increase as indicated by a straight line drawn through the main part of each curve. Here the solid dots represent the first brood of offspring that became gravid, the open circles the second brood.

Among the offspring from the Argonne Woods Pond the only comparable size in gravid fish was 41 mm. The 3 fish taken July 13 averaged 107 young per brood whereas the 4 individuals collected August 14 carried an average brood of only 16 embryos, a decrease of 91.

Further evidence of the decrease in productivity with increasing age is manifest by the decrease in the average number of young produced by fish of the same category in successive collections, despite the increase in the average size of the parent. In the Argonne Woods Pond (Table 11), the average size of the broods on June 9 was 205.4 for the parent females, which averaged 48.8 mm. in length. That average dropped to 155.7 young on July 13, when the parents averaged 52.6 mm., and the few parent fish remaining on August 14, averaging 52.3 mm., carried an average of only 42.7 embryos. Among the offspring from the Argonne Woods Pond the average number of young was 34.3 on July 13 and decreased to 15.6 on August 14, despite the fact that the average size of the mothers had increased from 35.3 to 42.2 mm. A similar perusal of the data from the Sanitary District Lake (Table 12) reveals that the average number of young carried by parent fish on June 9 was 30.4 and that it fell to 28.4 on July 13 and dropped still further to 8.8 on August 14, whereas the average size of the mothers on those dates was 38.8, 40.2, and 40.5 mm. In Parr's Pond (Table 13), where only gravid offspring were collected in numbers sufficient to warrant comparison, the average brood contained 44.5 embryos on July 31, 37.5 embryos on August 13 and only 17.1 embryos on September 19, although the respective lengths of the female were 31.6, 35.4, and 40.5 mm. Here it is obvious that, even though the mother fish actually increased considerably in length, the average size of the broods became markedly smaller as the fish grew older.

Many factors might have caused the observed decreases in the size of successive broods. Among the possible causes are: deterioration of natural conditions (water temperatures, chemical composition of the water, water level, availability of food, etc.); changes in population pressure; or some physiological change in the fish itself. An unfavorable change in the habitat seems unlikely, inasmuch as it would probably affect all fish alike, not only a selected group such as the gravid females. The effects of specific physical and chemical conditions on fecundity are not obvious. It may be that the size of successive broods decreased because of the liberation of large numbers of young early in the season with a consequent development of population pressure. This, however, seems unlikely as any compensation in the population due to increased numbers of individuals would probably take place after the young had been liberated and not in the reproductive mechanism. Physiological changes in the mothers appear much more likely to be the causative factors.

In the experiments referred to under the discussion of the length of the gestation period (see page 13)

the numbers of young in each of the broods born to each segregated female were counted. The data (Table 14) again show that the broods become smaller as the female becomes older. The second broods of 3 of the 4 females were slightly larger than the first whereas in the other individual the second brood was smaller than the first. The third brood was consistently smaller than the second. The last brood in the 2 fish that bore 4 broods was the smallest. The decrease in the size of these broods was certainly not the result either of a deteriorated habitat or of population pressure. The aquaria were kept clean and the young were removed soon after birth. Apparently the decrease in the size of the broods with increasing age of the mother fish is a physiological phenomenon closely associated with the production of young.

TABLE 14. Sizes of successive broods born to female mosquitofish under laboratory conditions, 1940.

Fish Number	NUMBER OF YOUNG			
	First brood	Second brood	Third brood	Fourth brood
1.....	24	28	7	..
2.....	34	36	12	6
3.....	42	47	18	8
4.....	21	16	3	..
Average.....	30.8	30.8	10.0	7.0

SENILITY

It is a well known fact that poeciliids, unlike many other fishes, have a definite period of sterile senility following reproduction. Evidence presented in the foregoing paragraphs shows that the period of reproduction in female mosquitofish, under natural conditions in Illinois, embraces only a single series of broods. Further, it has been pointed out that there is a marked decline in the reproductive efficiency of the female with increasing age; that is, the size of the broods becomes smaller as the reproductive period progresses.

The number of broods in the series cast by any individual depends on the age of that individual at first maturity and on the time of year at which reproduction began. A female becoming gravid during her first summer of life (age, 1 month) would bear, on the average, 2 broods of young, whereas an individual that did not begin to reproduce until the second summer of life (age, 8-10 months) would liberate approximately 4 broods. Further, the numbers of young per brood liberated by the females that reached maturity when only a month or so old were considerably smaller than those released by individuals that did not become gravid until the second summer of life.

The females that reached maturity during their second summer were obviously better suited physiologically to bear young than were those which reached maturity at an early age. The offspring in the

Argonne Woods Pond on July 13 were carrying fewer eyed embryos per brood on the average than were parent fish of the same lengths on May 19.

Senility among mosquitofish is not a function of age alone. Females that began to bear young when only a month or so old, apparently were physiologically more senile at an age of 4 months than were those which did not begin to reproduce until they were in their second summer of life. The older fish enjoyed a considerably longer and more fruitful period of reproduction than did those which reached maturity at an early age.

Further evidence of senility in female mosquitofish was found in the collections made in 1938. Among the fish taken in Pond 2 (Table 2), all individuals 33 mm. or more in length, with the single exception of the largest female (53 mm. long), were gravid. That non-gravid female was one of the parent stock (1 annulus on the scales) that apparently had completed her reproductive period. A microscopic examination of the ovary revealed no ova and the over-all aspect of the organ suggested that the fish was in a senile condition. Another female in similar condition was taken from the Argonne Woods Pond, August 14, 1939. It was a parent female 50 mm. long, and was not gravid, whereas all other females of comparable size or larger were carrying young (Table 9).

VALIDATION IN THE SIZE OF BROODS ACCORDING TO LOCALITY

The first indication of the relationship between the number of young per brood and the locality in which the mother fish lived was evident in the collections taken from 5 ponds near Chicago during August 1938 (Table 10, Fig. 7). Size for size the females in Pond 1 carried larger broods than did those from any of the other ponds. However, the largest brood, 174 eyed embryos, was taken from a female 54 mm. long from Pond 5. That fish and another female 57 mm. long from the same pond were known to be members of the parent stock (see page 7). It is likely that all other gravid females in the collection were young of the year. The reason for so great a diversity in the fecundity of the females in Ponds 1-5 is not obvious. The ponds were in the same general region and from all outward appearances were similar. It is unlikely that there could be any genetic cause inasmuch as the fish in all of the ponds came from the same stock and the plantings were made about the same time. Perhaps the most acceptable explanation is in the probability that the individual fertility of the ponds may have differed considerably.

More conclusive evidence that the locality or environment influences the fecundity of the female mosquitofish is shown by the data collected from the Argonne Woods Pond, the Sanitary District Lake, and Parr's Pond (Tables 11-13; Figs. 8-10). On June 9, the parent female from the Argonne Woods Pond carried an average of 205.4 embryos per brood whereas, on the same day, fish of the same age-

category from the Sanitary District Lake contained broods of only 30.4 eyed embryos. No collections were made from Parr's Pond until July 31. Although parent fish of the same length were seldom collected from the Argonne Woods Pond and the Sanitary District Lake on the same day, it is obvious that the fish in the former pond were much more prolific than those from the latter. Gravid parent fish 43 and 44 mm. long were taken from both ponds on June 9 and the mean size of the broods from the Argonne Woods Pond was 158 young as compared with 55.5 young from the Sanitary District Lake. The only parent female taken from Parr's Pond was 52 mm. long on July 31 and contained 154 eyed embryos. Fish of similar size were taken from the Argonne Woods Pond on July 13 and August 14 and carried broods of 155 and 30 young respectively on those 2 dates. Although such data are meager, it is evident that the parent fish in Parr's Pond were as prolific, if not more so, than those from the Argonne Woods Pond.

So far as the mature offspring in the 3 ponds are concerned, the fecundity of the fish in Parr's Pond was greater than that of either the Argonne Woods Pond or the Sanitary District Lake. The offspring in Parr's Pond not only liberated more broods of young (page 21), but the broods were larger than those from either of the 2 ponds near Chicago. Among the gravid offspring in Parr's Pond on July 31, the size of the broods ranged from 9 to 149 with an over-all average of 44.5 embryos, whereas in the Argonne Woods Pond on July 13 the number of embryos per brood among the offspring ranged from 8 to 107 with an average of 34.3 and in the Sanitary District Lake the offspring were not yet bearing young. On August 13 in Parr's Pond, the average number of young carried by offspring was 37.5 while on August 14 in the Argonne Woods Pond, the gravid offspring carried an average of 34.3 young and those in the Sanitary District Lake contained an average of only 5.3 young per brood. Furthermore, the offspring in Parr's Pond were still bearing young in October whereas no gravid fish were taken in the collections subsequent to August 14 in either of the 2 ponds near Chicago.

Comparison of brood sizes of offspring of similar lengths from these 3 ponds, where possible, clearly shows that the population of Parr's Pond was more prolific than either of the other two:

Mean sizes of broods from fish of similar size range:

Size range	Parr's Pond	Argonne Woods Pond
31-41 mm.	July 31	July 13
	58.6	34.3
41-44 mm.	Parr's Pond	Argonne Woods Pond
	August 13	August 14
	68.3	15.6
28-31 mm.	Parr's Pond	Sanitary District Lake
	August 13	August 14
	13.1	5.3

The differences in fecundity among the fishes of the 3 ponds mentioned above are to be attributed to

the marked differences in the fertility of those bodies of water. Parr's Pond supported an abundant crop of plankton, probably because of the continuous deposition of excreta by livestock pastured in the area. At the time the Argonne Woods Pond and the Sanitary District Lake were selected as sites for collections during 1939, it was felt that the former pond, because of the heavy growth of various aquatic plants, was considerably more productive.

Among the early investigators it was believed that the broods were rather small. Ryder (1882) suggested that the maximum size of a brood in *Gambusia* probably did not exceed 25 or 30 embryos and elsewhere (1883) stated that the number may be as few as 15 or 20. Smith (1912) recorded broods ranging from 85 to 134 fully developed embryos from females 45 to 50 mm. long taken in June 1912 from the Lower Potomac River and other broods of 18 to 30 embryos from fish of the same size from the same river in August 1890. His observations led him to suggest the production of more than 1 brood per season and that the second brood was smaller than the first because the presence of the developing embryos of the first brood inhibited the development of an equally large number in the later brood. Kuntz (1913) noted that the larger females usually gave rise to larger broods of young than did the smaller ones. He reported an average of 33 embryos per brood from a limited number of fish 50 to 60 mm. long from Beaufort, N. C., during the summer of 1912. The largest brood was 76 embryos. The size of the brood of the smaller fish ranged from 2 or 3 to about 20 individuals. The relatively small size of the broods was attributed to the fact that the fish were collected during the late summer and had probably passed the peak of their reproductive period. Seale (1917), working with aquarium-reared fish, recorded a maximum brood of 49 embryos.

In his early work on the mosquitofish, Hildebrand (1917) reported a brood of 63 embryos from a single female. Later (1921) he stated that the number of young produced at one time varies greatly, ranging from a few to a couple of hundred and listed broods of 120, 163, and 211 young. However, he said that the average number of young probably does not exceed 40, and that the number varied with the size of the female. In aquarium experiments he showed that a single female liberated as many as 345 young, consisting of 2 generations, during a single season.

In a rather comprehensive study of the fecundity of *Gambusia a. affinis*, Barney & Anson (1921b) reported broods ranging up to 226 embryos in a series of 800 females collected during 4 summers near Mound, Louisiana. The largest brood, 226 young, was carried by a female 43 mm. long, standard length (ca. 51 mm. total length). The smallest brood was probably from one of 5 fish 23-24 mm. long, standard length (ca. 31 mm. total length), that had an average of 8.6 embryos per brood although the actual size of the smallest brood was not given. The smallest gravid female observed was 17 mm. long, standard length

(ca. 22 mm. total length) and contained 9 embryos in August. Those workers combined the data collected from the various waters during the 4 summers into bi-monthly periods in an attempt to show the relationship between the fecundity and the length of the female. They noted

"... that fecundity in this species increases markedly as the length of the female increases. From the average count of embryos based on April and May observations, the fecundity of females of 2.3 to 2.4 cm. length is doubled when the fish is about 3.2 cm. long; the fecundity is about quadrupled when the length 3.5 to 3.6 cm. is reached. With each 2 millimeters' growth thereafter the fecundity is greatly increased."

The measurements given by Barney & Anson were standard lengths. They noted further

"... that in April and May the average number of embryos found in sixteen pregnant females was 108.1; for June and July, 60.3; for August and September, 40.5; and for October, November, December, January and February, 0; in fact, in these five months there were no embryos found in females of any size. . . ."

It is unfortunate that those workers failed to show the variation in size in successive broods from different populations and the possible differences in fecundity between the parent stock and the mature offspring.

De Buen & de Buen (1922), using fish that had been introduced into Spain for mosquito control, mentioned having found 38 advanced embryos in a female 46 mm. long. Artom (1924), who made his observations on fish transferred from Spain to Italy, counted broods of 15 and 34 embryos but did not indicate the sizes of the mother fish. Dulzetto (1928, 1934, 1935) in observing 78 parturitions among 29 mosquitofish living under conditions simulating those in nature, recorded broods that ranged from 4 to 97 embryos. The individual sizes of the mother fish were not given. Of the 29 individuals under observation by Dulzetto, 2 gave birth to only 1 brood, 9 bore only 2 broods, 14 bore 3 broods, and only 4 gave birth to 4 broods. The size of the first brood was usually smaller than the others and, in general, the second brood was the largest.

Sokolov (1936), working with *Gambusia* placed in rice fields in Central Asia as a mosquito-control measure, found that the fecundity depends on the size and age of the fish. He stated that mosquitofish are most prolific when they are 45-50 mm. in length which corresponds to an age of 2 or 3 years. Broods that ranged from 9 to 151 embryos were recorded but no mention was made of the individual lengths of the mother fish. Maksudov (1944) reported that there is a constant ratio between the weight of the mother fish and the weight of the offspring. He associated the increase in fecundity with the intensity of metabolism based on oxygen consumption. Prior to fertilization there is a greater oxygen demand because of the increased need for energy in the formation of yolk materia, etc., in the maturing ova. After fer-

tilization there is a sharp decrease in the demand for oxygen because the embryos use the food material stored in the mature egg.

The largest single brood of mosquitofish recorded in the literature was one of 354 young (Bonham, 1946). The fish carrying those embryos was taken from a small pond in Texas. Unfortunately, the size of the mother fish was not given.

LENGTH DISTRIBUTION AND GROWTH RATE OF MALES

Respective totals of 3,061 and 4,129 male mosquito-

fish were taken in 6 collections from each of the Argonne Woods Pond and the Sanitary District Lake during 1939 and a total of 3,351 males were collected in 4 samples from Parr's Pond in 1940. The mature males were distinguished from the immature individuals by a microscopic examination of the anal fin; those fish in which the formation of the gonopodium was complete were considered as mature and all others as immature.

Mature males were present in the last 5 collections from the Argonne Woods Pond, whereas such fish

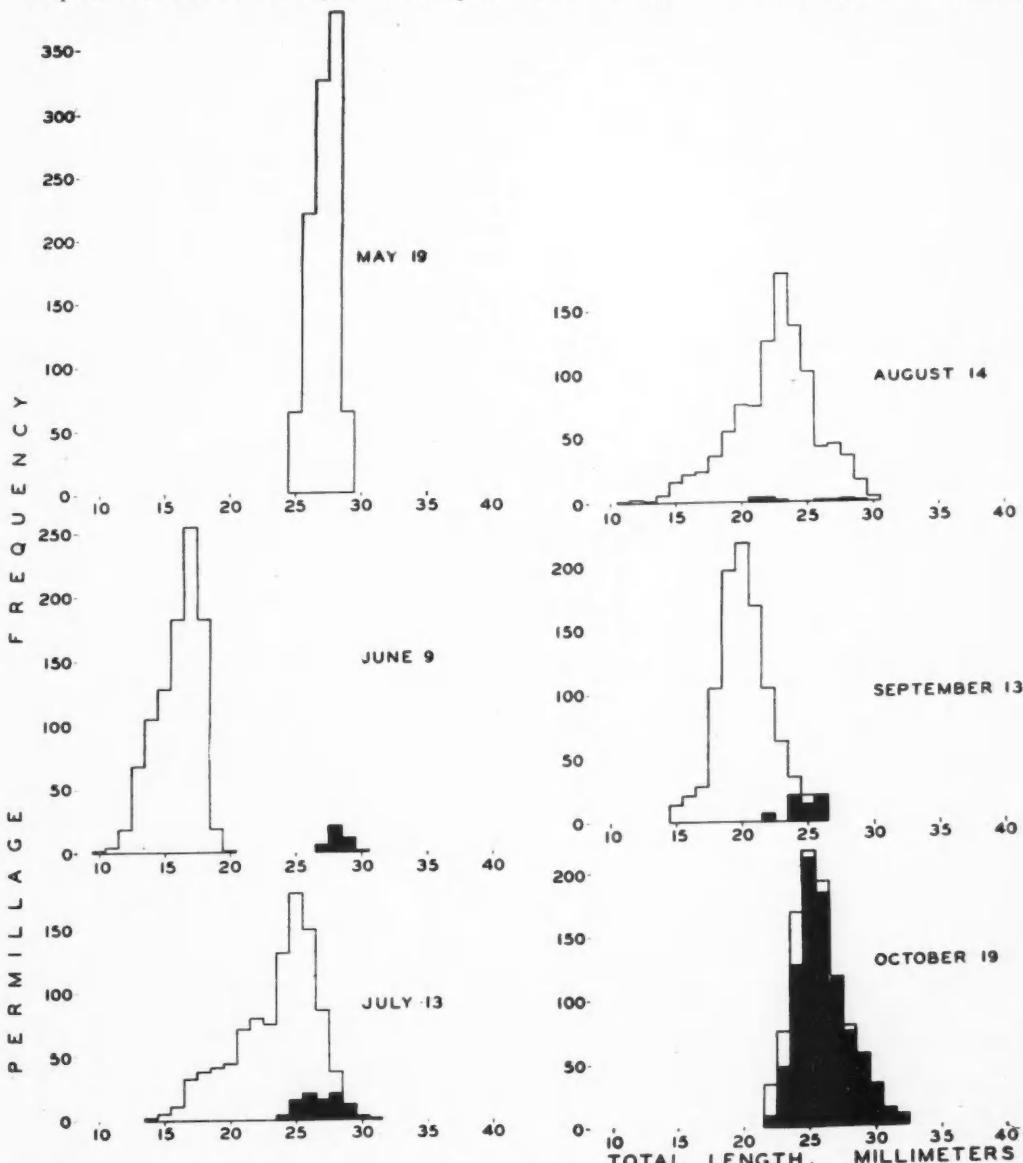


FIG. 11. Length frequencies of all male mosquitofish taken in each of six collections from the Argonne Woods Pond, Cook County, Illinois, during 1939. The shaded areas indicate the mature males, the unshaded the immature.

were present in all collections from the Sanitary District Lake and Parr's Pond. Immature males were present in all collections from the Argonne Woods Pond and Parr's Pond and were lacking only in the June 9 collection from the Sanitary District Lake. The size ranges and average lengths for all males in each collection are given in Table 15 for the Argonne Woods Pond, in Table 16 for the Sanitary District Lake and in Table 17 for Parr's Pond. These same

data are graphically represented in Figures 11, 12, and 13 respectively.

AGE AND GROWTH OF MATURE MALES

It has been shown by Turner (1941) that males of *Gambusia*, unlike the females which continue to grow until they die, stop growing, or grow only very little after the gonopodium has been completely formed.

The largest mature males taken in the collections

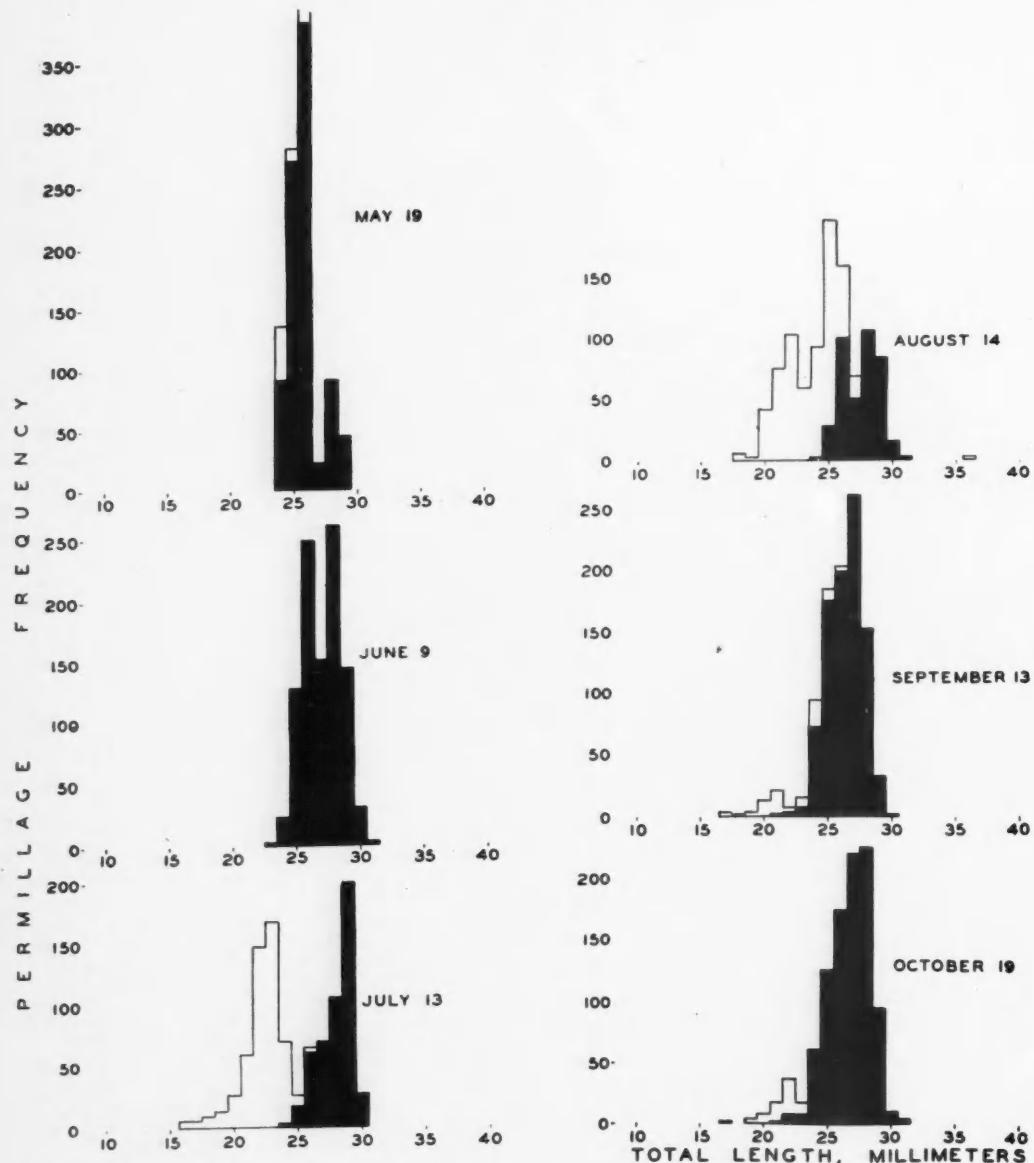


FIG. 12. Length frequencies of all male mosquitofish taken in each of six collections from the Sanitary District Lake, Cook County, Illinois, during 1939. The shaded areas indicate the mature males, the unshaded the immature.

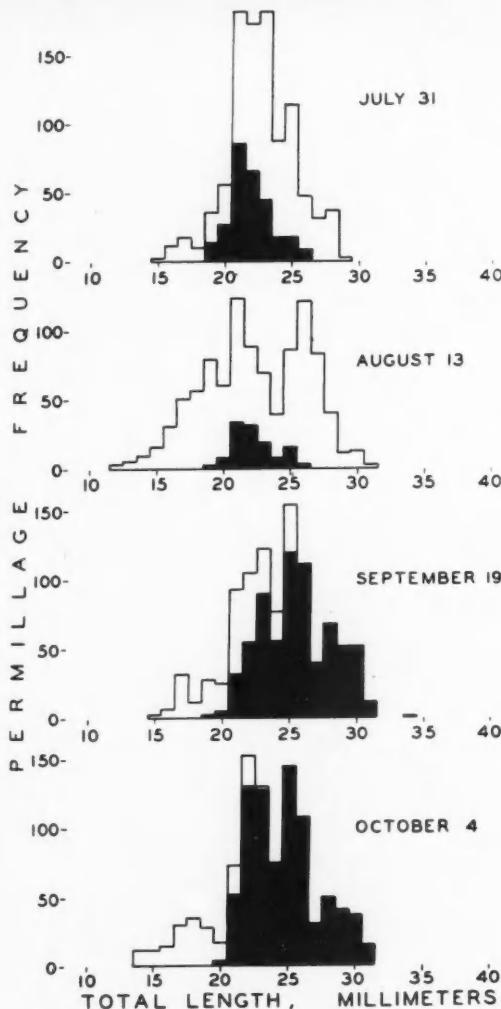


FIG. 13. Length frequencies of all male mosquitofish taken in each of four collections from Parr's Pond, Macon County, Illinois, during 1940. The shaded areas indicate the mature males, the unshaded the immature.

from the Argonne Woods Pond and Parr's Pond were 31 mm. long, whereas those from the Sanitary District Lake were 32 mm. in length. The largest mature male ever collected by the writer was 37 mm. long. That fish was taken from a small pond in Cook County, Illinois. The smallest mature males were present in the July 31 and August 13 collections from Parr's Pond (19 mm.).

Data from the May 19 collections from the Argonne Woods Pond and Sanitary District Lake show that some of the survivors from the preceding summer (1 annulus on the scales) were still immature. However, those fish had all reached maturity by June 9 and were disappearing from the population by the

TABLE 15. Numbers of mature and immature male mosquitofish showing minimum, average, and maximum total lengths in millimeters in each of six collections from the Argonne Woods Pond, Cook County, Illinois, 1939.

Date of Collection	IMMATURE			MATURE		
	Number	Length		Number	Length	
		Min.	Av.		Min.	Av.
May 19	19	25	27.2	29	—	—
June 9	646	10	16.0	20	27	27
July 13	839	14	23.2	29	86	24
August 14	1,087	11	22.5	30	12	21
September 13	133	15	20.0	25	9	22
October 19	24	22	23.7	28	188	22

TABLE 16. Numbers of mature and immature male mosquitofish showing minimum, average, and maximum total lengths in millimeters in each of six collections from the Sanitary District Lake, Cook County, Illinois, 1939.

Date of Collection	IMMATURE			MATURE		
	Number	Length		Number	Length	
		Min.	Av.		Min.	Av.
May 19	6	24	24.5	26	84	24
June 9	288	23	27.1
July 13	174	16	22.2	26	165	24
August 14	194	18	23.5	27	126	24
September 13	46	17	22.1	26	476	21
October 19	35	17	21.4	23	476	21

TABLE 17. Numbers of mature and immature male mosquitofish showing minimum, average, and maximum total lengths in millimeters in each of four collections from Parr's Pond, Macon County, Illinois, 1940.

Date of Collection	IMMATURE			MATURE		
	Number	Length		Number	Length	
		Min.	Av.		Min.	Av.
July 31	400	15	23.0	29	158	19
August 13	1,028	12	22.3	31	151	19
September 19	180	15	21.2	34	418	19
October 4	193	14	18.4	23	823	20

middle of August. None of the original planting of males in Parr's Pond were found in the collections.

Young-of-the-year males of the first broods which matured by July or August also died before the end of the summer. Such mortality of young-of-the-year males is clearly indicated in the data of the fast-growing populations of Parr's Pond and the Argonne Woods Pond but is rather obscure in those of the slow-growing population of the Sanitary District Lake. Thus it appears that the life span of the male mosquitofish is no longer than that of the female.

SIZE AND GROWTH OF IMMATURE MALES

If the rate of growth is constant for all individuals throughout the summer, and the formation of the gonopodium is a function of age alone, all males

would reach maturity at approximately the same length. Obviously such is not the case as indicated by the extent of the size range in the mature males from all collections. Some individuals grow faster than others before reaching maturity or else they mature later after having grown for a longer period of time. Such variations in length of time before maturity is reached are probably due to environmental factors such as availability of food, temperature, etc., or may be due to the physiological makeup of the individual fish.

The length-frequency distributions for the males in the Argonne Woods Pond (Fig. 11) indicate that some of the individuals which were immature on June 9 had become mature by July 13. Those fish which reached maturity during the interim had grown approximately 10 mm. as reflected by the change in their length distribution from 10 to 20 mm. on June 9 to one of 24 to possibly 31 mm. on July 13. Obviously not all of the immature individuals of June 9 became mature by July 13 inasmuch as there were large numbers of fish which were apparently members of the first brood of offspring that were still immature on the latter date. Data from the Sanitary District Lake (Fig. 12) indicate similar conditions although the rate of growth was not so rapid. Those fish which were immature on July 13 evidently did not attain maturity until September and the length distribution showed an increase, from 16-26 mm. on July 13 to 21-31 mm. on September 13, or about 5 mm. In Parr's Pond, where even more rapid growth of the population than that in the Argonne Woods Pond took place, the length distributions of individual broods were marked by the presence of other broods.

Evidence from the length frequencies of the male offspring which mature during their first summer indicated that the fast-growing individuals reach maturity at a younger age than do those which grow more slowly. The average size of the mature males taken in all collections from Parr's Pond (24.5 mm.) was considerably smaller than that for the Argonne Woods Pond (26.4 mm.) which in turn was smaller than that for the Sanitary District Lake (26.8 mm.).

Although immature males 34 mm. long were taken on several occasions, none were collected which had a greater total length than that of the largest mature male (37 mm.).

SIZE AND AGE AT FIRST MATURITY AND LENGTH OF THE REPRODUCTIVE PERIOD IN MALES

Inasmuch as male mosquitofish stop growing or grow only very little after the formation of the gonopodium is completed, the size of such fish at first maturity is closely approximated by the length at the time of capture. The considerable range in size of mature males of the same brood is explained by the fact that maturity is not reached by all individuals simultaneously. Turner (1941) found that some males became mature in 145 days (aquarium conditions) whereas others in the same group required 180 days.

The first mature male offspring were present in the same collection from the Argonne Woods Pond as the first gravid female offspring (July 13). All of these individuals were members of the first brood of young and consequently of the same age (6 weeks). The lengths of the mature male offspring ranged from 24 to 31 mm. (Table 15, Fig. 11) as compared with a length range of 31-41 mm. for the mature female offspring, all of which were carrying eyed embryos; a good indication that the individuals of both sexes had reached maturity as much as 2 weeks prior to the date of collection. The smallest mature males taken from the Argonne Woods Pond were 21 mm. long (August 14) and were probably members of the second brood of offspring. If that is the case, these smallest mature males were about the same age as those of the first brood when they reached maturity (4 weeks).

In the Sanitary District Lake mature male offspring were not collected until August 14 (Table 16, Fig. 12), whereas the first mature female offspring had been taken a month earlier (July 13). It appears that the mature males in the collection of August 14 might have been fish which survived from the preceding summer but examination of the scales showed that a few individuals were young of the year (no annulus). Most of the male offspring of this group, however, did not become mature until sometime before September 13 inasmuch as they were present as mature fish in the collection of that date. Here it is apparent that the males required a longer period of time to reach maturity than did the females. Perhaps this was due to a delayed development either because of a slower rate of growth, as exhibited by the rest of the population, or the lack of necessity to become mature when there was already an abundance of mature males present. The first mature male offspring were at least 8 weeks old by August 14 although they might have reached maturity at any time between that date and the date of the previous collection (July 13). However, the bulk of the group was immature on August 14 but had reached maturity by September 13 and so were somewhere between 8 and 13 weeks old when they became mature. The first mature male offspring collected from the Sanitary District Lake were not present in the samples until August 14, a month after similar fish had been taken from the Argonne Woods Pond. It is not meant to infer here that the fish in the Argonne Woods Pond reached maturity a month earlier than those of the Sanitary District Lake (the latter could have become mature at any time during the interval) but rather that the fish in a more slowly growing population required a greater length of time to reach maturity.

In Parr's Pond there was an abundance of mature male offspring when the first collection was made on July 31 (Table 17, Fig. 13). The identity of the brood or broods to which these individuals belonged is obscure as both parent fish and offspring were liberating young at the time (see page 19). In the

length distribution of the female offspring of the same collection, the modes indicate the presence of 2 broods (Table 8). The presence of very small mature males (19 mm. total length) together with a corresponding size range for such fish in each of the first 2 collections indicates that the males were reaching maturity at a rather constant size and rate. Also it is apparent that those individuals were reaching maturity at an early age as reflected by the length frequencies in successive collections. In such a rapidly growing population some of the immature males taken on July 31 had reached maturity by August 13 and some of those of the August 13 collection had become mature by September 19. However, it is clearly shown by those same length frequencies that all of the young-of-the-year males did not reach maturity at the same age. There were immature males present on July 31 larger than the largest mature males taken on August 13. It may be that (1) these larger fish had become mature and disappeared from the population during the interim, or (2) they had remained immature until later on, as fish of a comparable size were present in the collection of September 19. The latter hypothesis seems doubtful in the light of the findings from the Argonne Woods Pond and the Sanitary District Lake where males in the more rapidly growing population apparently reached maturity at an earlier age and died a short time after becoming mature.

Earlier workers have shown that in *Gambusia* one fertilization is sufficient for a female to develop and liberate a series of separate broods. Apparently the majority of the male offspring in the Argonne Woods Pond (Table 15, Fig. 11) reached maturity by October 19, but the absence of mature males in the collection of May 19 indicates that they did not survive to the latter date. Although no mature males were present in the May 19 sample, certainly such fish had been present before that time inasmuch as all of the females in that collection were gravid (Table 6, Fig. 2). It seems reasonable here to assume that some of the male offspring which had reached maturity the previous fall survived the winter and died after fertilizing the females early in the spring. Also it is obvious that some individuals remained immature over winter and attained maturity the following spring after the fertilization of the females had taken place. These late-maturing fish were not present in the samples after July 13. After that date young-of-the-year mature males served the function of fertilizing the female offspring as they became mature.

In the Argonne Woods Pond some of the immature males of the June 9 collection as indicated by the length frequencies (Fig. 11), had matured by July 13 but the numbers present as mature fish on the latter date were considerably fewer than the immature fish present a month earlier. The presence of large numbers of immature fish on July 13 indicates that many of the fish which were immature on June 9 may not yet have matured by July 13. However, the mode in the length distribution of the immature

fish (25 mm.) on July 13 as compared with the mode on August 14 (23 mm.) indicates that the larger fish of July 13 had become mature and disappeared from the population within the space of a month. The mature males were considerably fewer than the immature individuals in all collections from the Argonne Woods Pond with the single exception of the sample taken in October. As shown previously, the males reached maturity at about the same or a little greater age than the females. Thus, if the mature males were as long-lived and had reached maturity at about the same age as the gravid females, they should have been present in as great or greater numbers than the immature males in the collections taken early in the summer. This, however, was not the case in the Argonne Woods Pond or either of the other 2 ponds. It was not until September or October, as the case in each pond may be, that the numbers of mature males equaled or exceeded that of the immature males of a size greater than that of the smallest mature male, whereas among the females, the number of gravid individuals were predominant throughout the reproductive season.

The evidence here presented indicates that males, although they reach maturity at about the same or a slightly greater age than the females, do not live so long after reaching maturity. This age after maturity among males varies according to the season at which the individual fish become mature. Those fish which mature in the fall may or may not live on as mature individuals until early the following spring, an interval of 7 to 8 months, whereas those of the first and possibly the second broods which mature during the same summer in which they were born probably do not live more than a few weeks after maturity has been reached. Furthermore, inasmuch as one fertilization is ample for a female to produce a complete series of broods, and the females die after one such period of reproduction, it seems reasonable to assume that males, after having contributed their bit to the reproductive process, should also disappear from the population as soon as the females have been fertilized.

There is no cycle in the reproductive process of male mosquitofish. Copulation takes place whenever the opportunity presents itself and mosquitofish are quite promiscuous in their breeding habits. Turner (1937) has pointed out that the production of sperm is practically continuous after the attainment of sexual maturity.

SEX RATIO AND DIFFERENTIAL DEATH RATE IN *GAMBUSIA*

The sex ratios for the mosquitofish, as reported in the literature during the first quarter of this century, indicated a disproportionate preponderance of females over males. Those ratios were perhaps inaccurate for two reasons: (1) the sole criterion used to distinguish sex was the presence or absence of the modified anal fin (gonopodium) and, (2) the gear used was very selective in that the seines or dipnets

were usually of such coarse mesh that only the relatively large specimens (mostly females) were captured. Also it is known, as will be discussed later, that there are considerable variations in the sex ratios in collections from the same body of water at different times of the year. In only a few instances has the writer found reference to a great preponderance of males in a collection.

Smith (1892) reported 68 females and one male in a single collection from the Lower Potomac River in Maryland and a ratio of 3 females to one male in collections from the Pasquotank River in North Carolina (Smith 1893). Fowler (1910) found males of *Gambusia* equally abundant with females in New Jersey waters. Hildebrand (1917) stated that the sex ratio in *G. a. holbrookii* in collections from Georgia was 8 or 9 females to one male. Barney & Anson (1921a) reported ratios of 5.8:1 and 4.5:1, females to males, in collections from Louisiana in 1918 and 1919 respectively. Those authors used the term "male" to indicate only fish in which the gonopodium was well developed; "young" fish were all those under 1.5 cm. standard length (ca. 21 mm. total length) with the exception of a few individuals which exhibited a modified anal fin; and "females" were all fishes more than 1.5 cm. standard length without any modification of the anal fin. This last group must have included many immature males. Hildebrand (1917) and Turner (1941) have called attention to the fact that the gonopod develops gradually, and at no definite age or length of the fish. In my 1939 collections, for example, some males were mature at 21 mm. and others were still immature at 30 mm.

Geiser (1924), in rearing 94 newly born mosquito-fish (four complete and separate broods) to an age when the sex could be distinguished with certainty, was first to establish a 1:1 ratio (46 males, 48 females) between the sexes in this fish at birth.

Hildebrand (1927), in a study in which the sex of each individual was determined by a microscopic examination of the gonads, found the sex ratio to be 1:1 (1,295 females and 1,298 males) in "immature" mosquito-fish less than 20 mm. long; a ratio of 1.48:1 (1,233 females and 712 males) in immature fish larger than 20 mm.; and a ratio of 4.4:1 (83,554 females and 19,596 males) in mature specimens. Those data were based on a large number of collections, taken in a great variety of waters representing nearly every environment in which *Gambusia* lives, over a period of five years (1921-1926). As late as 1927, however, Jordan stated that at birth the sex ratio in *Gambusia* was 10 females to one male.

In four ponds from which de Buen (1930) made collections in 1928 and 1929, he found ratios of 253, 136, 153, and 388 females to 100 males. In all collections he used the presence of the gonopod as the criterion for distinguishing the sexes, and divided the fish into size groups similar to those of Barney & Anson (1921a). However, de Buen made no attempt to sex fish that were less than 20 mm. in length. Dulzetto (1934, 1935), in studying the sex ratio in

31 individual broods of *Gambusia* in 1934, found variations in the ratios among the broods from 37.5 to 400.0 females per 100 males but the sex ratio in all broods combined was very close to 1:1 (672 males, 661 females). During 1935, in a repetition of the 1934 study, Dulzetto found variations from 16.6 to 240.0 females per 100 males in 24 broods and again there was an over-all average of 1:1 (435 males, 443 females). The broods utilized in this study by Dulzetto were consecutive broods from 11 females in 1934 and 10 females in 1935.

Barnickol (1941) and Rice (1941) observed identical ratios of 7 females to one male in 1939 and 1940 respectively in mosquitofish taken from Reelfoot Lake, Tennessee.

There were considerable differences in the sex ratios in the 5 preliminary collections made by the writer near Chicago in August 1938. In those collections, females outnumbered the males in 3 samples whereas males were present in larger numbers in the other two. The length frequencies and numbers of individuals of each sex for each of the 5 collections, indicating a range of 26 to 652 females per 100 males, have been given in Table 2. In the combined samples the ratio was 190 females to 100 males (743 females, 391 males). The wide variation in these sex ratios might have been due to the small numbers of specimens in the collections. Segregation of the sexes which caused the individuals of one sex to be captured more easily than those of the other, also may have played a role in these and other collections discussed later.

In an effort to obtain further information regarding sex ratio in *Gambusia*, the entire collections of 1939 and 1940 were sexed. Furthermore, in 1940, newborn individuals of complete broods were separated from each other and allowed to reach maturity in isolation.

During the entire study a total of 19,150 female and 10,943 male mosquito-fish were sexed. This shows an over-all sex ratio of 175 females to 100 males in a total of 21 collections taken during 3 summers (1938-1940). The numbers of topminnows of both sexes and the sex ratio (in parentheses) of the fish in each collection taken from the three ponds in Illinois during the summer of 1939 and 1940 are given in Table 18. From those data it is evident that the sex ratios differ considerably among individual collections from the same pond at different times during a single summer. The greatest difference in sex ratio was exhibited in the collections from the Sanitary District Lake during 1939. In those collections the ratios ranged from 2 to 537 females per 100 males, an extreme variation of 535. In the samples from the other two ponds the differences in sex ratio were not nearly so great—170 to 296 females per 100 males in Parr's Pond during the late summer of 1940. There appears to be an irregular increase in the frequency of females during middle and late summer. Such a shift might be explained by a differential death rate in the two sexes.

TABLE 18. Total numbers of male and female mosquitofish collected from ponds in Illinois, 1939-1940. The figures in parentheses indicate the sex ratio.

SANITARY DISTRICT LAKE COOK COUNTY, ILLINOIS			ARGONNE WOODS POND COOK COUNTY, ILLINOIS			PARR'S POND MACON COUNTY, ILLINOIS		
Collection date	♂♂	♀♀	Collection date	♂♂	♀♀	Collection date	♂♂	♀♀
May 19, 1939	89	2 (100:2)	May 19, 1939	19	39 (100:205)	July 31, 1940	553	494 (100:89)
June 9, 1939	284	71 (100:25)	June 9, 1939	670	1,395 (100:208)	August 13, 1940	1,172	1,042 (100:89)
July 13, 1939	328	1,538 (100:469)	July 13, 1939	926	1,577 (100:170)	September 19, 1940	601	920 (100:153)
August 14, 1939	322	1,728 (100:537)	August 14, 1939	1,109	2,218 (100:200)	October 4, 1940	1,036	1,144 (100:110)
September 13, 1939	1,541	2,244 (100:146)	September 13, 1939	144	373 (100:259)			
October 19, 1939	1,439	2,973 (100:207)	October 19, 1939	211	624 (100:296)			
Total	4,003	8,556 (100:214)		3,079	6,226 (100:202)		3,362	3,600 (100:107)

The sex ratios of the combined collections from each of the 3 ponds indicate that the relative abundance of the females is greater in standing populations than in newly populated ponds. The combined collections from each of the 2 ponds which supported well-established populations were roughly 200 females to 100 males, whereas in Parr's Pond the sex ratio in the combined collections was very nearly 1:1.

Laboratory experiments performed by the writer at the University of Illinois showed that the sex ratio of *Gambusia* at birth approximates 1:1. Newly born young of 4 entire broods (broods 1, 2, 3, and 4 in Table 19) were separated from each other and kept isolated, after the method of Geiser (1924), until maturity was reached. Also each of 2 entire broods (broods 5 and 6 in Table 19) of new-born young were placed in aquaria and allowed to grow to maturity in the presence of the other individuals. When the fish were thought to have reached maturity, the anal fin of each individual was inspected under the microscope for determination of sex. The date of birth, the date on which the fish were sexed under the microscope, the number of males and females and the sex ratio of each of the 6 different broods are shown in Table 19. Those data indicate a 1:1 sex ratio for *G. a. affinis* at birth.

If the sex ratio is 1:1 at birth, the preponderance of females later in life must be attributed to the differential death rate of the sexes. The literature on this subject is not extensive. Barney & Anson (1921a) found, in shipping several cans of *Gambusia* on a railroad journey of about 24 hours during August, that a large number of females but none of the males died. From this observation they stated that it was evident that male mosquitofish are somewhat more resistant to higher temperatures than are the females.

Geiser (1921, 1924), however, showed conclusively that the death rate for males of *Gambusia* in transit was greater than that of the females regardless of whether the temperature was high or low. In ship-

ments made during cold weather (November) the death rate of males was about one and a half times that of the females, whereas in shipments made during warm weather (March) the males died at approximately two and a half times the death rate for the females. This greater death rate occurred in the males even though many of the females were heavy with young in March. Furthermore, Geiser (1924) indicated that female mosquitofish are more resistant to the rigors of winter than are the males. Of 100 fish, fairly equally divided as to sex, that were left outdoors over winter, there were 32 survivors the following spring, of which only one was a male.

Hildebrand (1927) stated that, in a total of 41,073 adult fish, consisting of 7,357 males and 33,736 females, used in a series of experiments, there was a mortality of 29.75 per cent among the males and only 22.33 per cent among the females. These experiments include the study of the effects of high temperatures,

TABLE 19. Number of male and female mosquitofish indicating sex ratio in broods raised from birth (1940). The fish from broods 1-4 were segregated and the fish from each of broods 5 and 6 were raised together.

Brood number	Date of examination	Number of males	Number of females	Date of birth	Sex ratio (100♂:♀)
1.....	IX:15	13	12	VII:30	100:92
2.....	IX:22	15	16	VIII:12	100:107
3.....	XI:28	10	11	IX:17	100:110
4.....	XI:28	15	14	X:19	100:93
Total or average		53	53		100:100
5.....	XI:28	19	20	VIII:4	100:105
6.....	XI:28	13	11	IX:6	100:85
Total or average		32	31		100:97
Total or average		85	84		100:99

of over crowding the fish, and of using varying amounts of water in which to hold the fish. Hildebrand admitted that in some individual lots proportionately fewer deaths occurred among the males than among the females but stated that generally the reverse was true.

Although no special experiments on the resistance of Gambusia to adverse conditions were performed, many observations were made while transporting many thousands of those fish in Illinois and Michigan. It was noticed that mosquitofish were easier to transport over long distances than were most fish native to the area. In July 1944, 165 Gambusia of various sizes were placed in a 10-gallon milk can, approximately half full of water, in the trunk of the writer's automobile. These fish were the remnant of a group being planted for mosquito control. They remained in the can for a period of 9 days during which time they travelled a total of 782 miles. The water was not aerated nor were the fish given any food. Air temperatures reached into the nineties and certainly the temperatures in the car trunk might have exceeded 100 since no attempt was made to keep the car in the shade. Every other day the fish were observed to find out how they were getting on. At the end of 9 days the fish were removed from the milk can and in that period only 3 fish (all adult males) had died. The remaining 162 fish were sexed and if they were obviously males or females were liberated in a pond near Ann Arbor. Among the 162 fish there were 46 in which the sex was not readily recognizable. These were preserved and the sex determined under a microscope. The sex ratio of the entire lot was 166 females (103 fish) to 100 males (62 fish, of which 3 died during the experiment).

The experience of the writer has shown that in the great majority of cases male mosquitofish are much less resistant than are the females. On October 4, 1940, after a sample of mosquitofish from Parr's Pond was preserved, 7,750 fish were placed in four 10-gallon milk cans and taken to the North Pollywogs near Danville, Illinois, a distance of about 90 miles. The water in which the fish were carried was not aerated. At the end of the journey, the live fish were planted out and the dead ones were preserved. A total of 897 fish perished en route and of these 634 (70.7 per cent) were males and the remainder were females. Inasmuch as the sex ratio of the preserved collection was 110 females to 100 males it may be assumed that the ratio of the transported fish was similar.

In July 1944, Mr. Aden C. Bauman of the Missouri Conservation Commission took 149 Gambusia, from stock which had survived 3 winters in Michigan, to Columbia, Missouri. Those fish, some of which were gravid females, were placed in about 4 gallons of water in a can in the trunk of Mr. Bauman's car. The following is an excerpt from Mr. Bauman's letter of February 12, 1945: ". . . Returning to Columbia after a 2-day stop in Illinois, I had 137 live Gambusia. I kept them iced while in Illinois, at which

time they were kept in a cellar. Twelve Gambusia, all adult males, died in transit. . . ."

It has been pointed out earlier that male mosquitofish, although they become mature at about the same age as the females, do not have so long a reproductive period. In the populations studied here the mature females were apparently longer lived than the mature males of the same brood. The females lived on to liberate several broods of young whereas the males, after fertilizing the females, disappeared.

In the considered opinion of the writer, Gambusia is easily transported from one place to another and, although both sexes are highly resistant to adverse conditions, the females, even though heavily gravid, are more resistant than are the males. The mature males, having a higher death rate than the females, are less numerous in long standing populations with the result that unequal sex ratios occur in adult stocks.

ACCLIMATIZATION OF GAMBUSIA TO NORTHERN ILLINOIS AND MICHIGAN

The natural range of the western mosquitofish has already been discussed (see page 5). Krumholz (1944) gave a short summary of the acclimatization of that poeciliid into northern Illinois and southern Michigan.

The first introduction of Gambusia into northern Illinois took place in 1923 when some fish, taken from a small pond on the campus of the Southern Illinois State Normal University at Carbondale, were transferred into a garden pool on the estate of Mr. Roland D. Whitman in Winnetka. That planting was augmented by another in 1925. In their new environment, near Chicago, the fish were subjected to much more rigorous climatic conditions than those to which they had been accustomed at Carbondale (Holeomb, 1941). However, Jordan (1927) stated that the director of the Illinois pond at Carbondale reported that a foot and a half of ice over the pond surface did not kill the mosquitofish as they were all hibernating in the bottom of the pond. Winters of this severity, although infrequent in the region of Carbondale, apparently aided the indigenous population of Gambusia in becoming harder than other members of the same species farther south.

Evidently a considerable amount of good fortune accompanied the success of the plantings in Winnetka in 1923 and 1925. Clarke (1939), among the first to suggest the use of fish for mosquito control in the Chicago Region, stocked ponds on seven golf courses with mosquitofish from Carbondale in 1928. None of these fish survived the subsequent winter, so another attempt to introduce the fish from southern Illinois was made in 1929. Again the plantings failed, and in 1933 Clarke drew upon the stock in Winnetka for the first time and introduced the fish into 7 ponds in suburban Chicago in the hope of establishing hatcheries as a source for widespread stocking in later years. The method of dispersal proved satisfactory

and since that time more than 100 waters of various types in Cook County, Illinois, have been stocked with these "native" mosquitofish.

Plantings of southern Gambusia failed in the vicinity of Camden, New Jersey, and Philadelphia as will be shown later (page 35). Herms and Gray (1940) stated that Gambusia had been successfully introduced into the Puget Sound area, but the winters there, although farther north, are much more temperate than in the region of Chicago.

The western mosquitofish was first introduced into Michigan on June 30, 1941, when, through the courtesy of the Michigan Institute for Fisheries Research, several thousand specimens were transferred from the Argonne Woods Pond, Cook County, Illinois. These fish were taken to the State Fish Hatchery at Drayton Plains, Michigan, and during the course of the summer were introduced into a number of natural and artificial bodies of water. It seems unnecessary to describe each of these ponds other than to mention that 3 of the natural lakes were dystrophic, one was an acid bog, and one an eutrophic lake. The artificial ponds ranged in age from a few years to two decades and were all mud-bottomed with good growths of aquatic plants. All but 2 of the ponds were inhabited by other species of fish. Goldfish were introduced into one of the ponds sometime during the late fall of 1941 and in October 1944, 25 largemouth black bass fingerlings were placed in the pond.

The topminnows have successfully passed 5 winters in 2 of the artificial ponds. The other artificial ponds were not permanent. It is not known whether the plantings in the natural waters were successful although no topminnows have been collected from any of them.

Since 1941, mosquitofish have been introduced into natural waters in Michigan as far north as the Straits of Mackinac. Considerable numbers of Gambusia have escaped into the waters of the Clinton and Huron River systems from ponds which empty into those waters. The escaped fish may eventually establish populations in Lake St. Clair and the Lake Erie marshes. No adequate surveys of these lakes or streams have been made since the introductions of the mosquitofish and it is not known whether the fish have become established.

In 1942, some of the stock which had survived the preceding winter near Ann Arbor were sent to Dr. C. M. Breder, Jr., then Director of the New York Aquarium, in an attempt to get the species established in the region around New York City.

The fish which Mr. Bauman took to Missouri in July 1944 (page 32) were placed in three small shallow ponds in the vicinity of Columbia. An excerpt from Mr. Bauman's letter of February 14, 1945, follows: "By freezing weather the Gambusia had reproduced until there were 'swarms' of them in all ponds. The freeze-up came December 1, 1944, and was followed by a 10-inch snow on December 10. By January the ice was 8-10 inches thick. On February 12, the ice had left our ponds and streams and we can

say (with reservations) that spring is here. Also I can report that the hardy Gambusia from Michigan came through one of our hardest winters in years, whereas our river-bottom Gambusia always died when brought into this vicinity."

The minimum requirements necessary for the mosquitofish to pass a winter successfully in northern Illinois or southern Michigan, where there is a more or less continuous ice cover on the ponds during the winter months, are not easily defined. Where introduction of this fish into northern waters are contemplated, the first requirement is the selection of a brood stock from a region where climatic conditions simulate those of the new habitat as closely as possible. The brood stock should be obtained from the northern limit of the natural range of Gambusia or at least from a point where there is an ice cover on the ponds at some time during the year. The fish which were introduced into and became established in the Chicago region, were brought from Carbondale, Illinois. Although these fish did not come from the extreme northern limit of their natural range, freezing temperatures and ice formation occur during the winters and it may be assumed the fish were much more resistant to cold than fish brought in from a southern state. Perhaps the introductions in the vicinity of Philadelphia might have met with greater success if the introduced stock had come from the Chesapeake Bay area rather than from North Carolina.

The next problem is the selection of the proper type of pond in which to place the fish that it is desired to carry over winter. It has been the experience of the writer that the fish have a better chance of survival if they are introduced into a soft-bottomed pond, having a maximum depth of at least five feet, and free from other fish. The fish should preferably be placed in their new environment early in the summer so that the population can become well established before the ensuing winter. In some instances Gambusia will survive the winter in ponds of a lesser depth than five feet. The mosquitofish which had been left in Sam Parr's Pond, Macon County, Illinois, in the fall of 1940, survived the subsequent winter in water which had a maximum depth of 10 inches including an ice cover of about 4 inches during part of the winter.

In making the introductions of this exotic species into Michigan, ponds of different types, such as natural lakes with standing fish populations, bog ponds which harbored a few species of small fishes, impoundments with and without other fishes present, and hatchery ponds, were selected. Collections made the following spring showed the mosquitofish survived in all but one of the artificial bodies of water. One of the impoundments, a spring pond, contained brook trout (*Salvelinus f. fontinalis*) which had spawned successfully in the outlet stream. The floor of that pond supported a dense growth of Chara and the mosquitofish established themselves and successfully passed 3 winters in the pond. It is not known

whether the population is still present. In another impoundment there was a large population of sticklebacks (*Eucalia inconstans*) and the topminnows have become well established there. In still another pond, formed by excavating an intermittent marsh, *Gambusia* have withstood competition from large numbers of goldfish (*Carassius auratus*) and the population has increased so greatly that it has become a source of supply for further plantings since 1941.

The hatchery ponds, although often contaminated with various species of fish as yellow perch (*Perca flavescens*), black crappies (*Pomoxis nigro-maculatus*), pumpkinseeds, sticklebacks, minnows (*Notropis heterodon* and *N. heterolepis*) and other species, have supported *Gambusia* through 3 winters and have furnished a good source of supply for further introductions. Although these hatchery ponds have maximum depths of only 3 or 4 feet, there is a continuous flow of water through them during the winter which prevents the accumulation of harmful products of decomposition.

Gambusia have survived winters under the ice in northern Illinois in ponds having hydrogen-ion concentrations (pH) which ranged from 6.6 to 7.8 and total hardness (methyl orange alkalinity) readings between 12.8 p.p.m. and 320.4 p.p.m. Also mosquitofish have failed to survive the winter in ponds more than five feet deep and whose pH and total hardness were well within the above range. Thus, although the range of tolerance to pH and total hardness of water shown by *Gambusia* is fairly wide, there are undoubtedly other factors which directly affect their ability to maintain a population.

In placing *Gambusia* in ponds where larger predaceous fish are present it is imperative that there is sufficient shallow water and aquatic vegetation to furnish ample cover.

Mosquitofish are capable of maintaining population in salt, brackish, or fresh water. Along the Gulf Coast they are found in all three of these habitats. Sieault (1934), in studies on the ability of *G. a. holbrooki* to become adapted to salt water in Morocco, found that it was possible to transplant the fish from fresh water into water, to which 12 grams of sodium chloride per liter had been added, without any apparent diminution of their larvivorous capacity. Also he found that by progressive adaption it was possible to transfer the topminnows from fresh water into water which approximated sea water in salt content (33 grams, sodium chloride per liter). In concentrations greater than 35 grams of sodium chloride per liter the *Gambusia* died.

GAMBUSIA AS AN AGENT FOR MOSQUITO CONTROL

That fishes are of value in mosquito control has been recognized since the latter part of the nineteenth century. An article which appeared in *Insect Life* (1891, 4:223) told of an English gentleman, who, having discovered that the mosquitoes which troubled him bred in large tanks kept for the purpose of

storing fresh water, placed a pair of carp in each tank and succeeded in eliminating the insect pest.

The renowned entomologist, Leland Ossian Howard (1901), after obtaining information on the feeding habits and larvivorous capacity of *Gambusia* from Mr. W. P. Seal, Dr. H. F. Moore, and Dr. H. A. Veazie, was first to advocate the use of that poeciliid for the control of mosquito larvae. At that time, however, Seal, seemed inclined to believe that the pumpkinseed, *Lepomis gibbosus* (L.), a small sunfish, would be more satisfactory because its spiny rays would make it less likely to be eaten by larger fishes. Smith (1904) stated that no fish native to New Jersey could control mosquito larvae as efficiently as the mosquitofish and requested Seal to introduce *Gambusia* into that state. Accordingly, Seal (1910) transported 10,000 *Gambusia* and *Heterandria* from North Carolina to New Jersey and liberated them in the vicinity of Camden in 1905 (Howard 1910). This was the first attempt to introduce *Gambusia* into northern waters as a mosquito-control measure.

At the request of Mr. D. L. Van Dine, entomologist of the Hawaii Agriculture Experiment Station, Jordan sent Seale (1905) to Texas where the latter obtained fishes of 3 genera (*Fundulus grandis*, *Gambusia affinis*, and *Mollienesia latipinna*) in order to ascertain their effectiveness in the control of mosquito larvae. *Gambusia* proved to be the most efficient, but Seale took about 150 of each of the 3 kinds to Honolulu where they were again tested with similar results. That was the first time *Gambusia* was taken outside the continental United States for use in mosquito control. In 1913 Seale (1917) carried 24 mosquitofish in a glass jar in his stateroom from Hawaii to the Philippine Islands. In both island groups the topminnows have thrived and reproduced in large numbers.

Jordan (1926, 1927) was highly instrumental in having mosquitofish introduced into other countries. The American Red Cross and the International Health Board of the Rockefeller Foundation undertook a major portion of the responsibility of having such shipments made.

The introduction of *Gambusia* into Europe was due also to the efforts of Dr. Massimo Sella who, with the aid of the Red Cross, arranged to have shipments of *G. a. holbrooki* sent from Augusta, Georgia, to Spain and Italy in 1921. The lot sent to Spain arrived in good condition and the fish were placed in natural waters at Talayuela, Cáceres (de Buen and de Buen 1922), and thence transferred to other parts of the Iberian Peninsula. The fish intended for Italy failed to survive the trip but the following summer Sella (1926) took some of the stock from Spain to Italy. Because the mosquitofish from this original introduction were unable to withstand the winters in northern Italy, a shipment of *G. a. affinis* was sent from Carbondale, Illinois, to Trieste in November 1927 (*Rivista die Malariaologia*, 6:999-1000).

Both of these subspecies of *Gambusia* have been widely distributed in Europe, Asia Minor, Africa,

and nearby islands. Because of the obscurity of some statements in the literature regarding the introduction of mosquitofish it has been impossible to separate the introduction according to subspecies. A list of places where *Gambusia affinis* is known to have been introduced in the Old World follows:

Albania	Greece	Romania
Algiers	Hungary	Russia
Austria	Italy	Sardinia
Bulgaria	Jugoslavia	Sicily
Caucasus	Macedonia	Spain
Corsica	Madagascar	Syria
Dalmatia	Mauritius	Tripolitania
Egypt	Morocco	Turkestan
Fezzan	North Caucasus	Turkey
France	Palestine	Ukraine
Georgia	Portugal	Union of
Germany	Reunion	South Africa
	Rhodes	

On the other side of the world, the mosquitofish has been distributed over a much greater area. Following its establishment in the Hawaiian and Philippine Islands, *Gambusia* has been introduced into the following:

Alaska	Cook Islands	Japan
Bonin Islands	Federated Malay	Marianas Islands
Borneo	States	New Ireland
Burma	Fiji Islands	New Guinea
Caroline Islands	Formosa	New Zealand
Celebes	Guadaleanal	Samoa
Ceylon	Hongkong	Siam
China	India	Tahiti

During World War II the United States Army has introduced mosquitofish into various island groups in the Pacific war theater not mentioned here.

In the Western Hemisphere the mosquitofish has been introduced into Ecuador and Argentina in South America; Porto Rico, the Virgin Islands and other of the Lesser Antilles; and into Arizona, California, northern Illinois, Kansas, Massachusetts, Michigan, Nevada, New Jersey, New Mexico, Ohio, Utah, Washington, Wisconsin, and probably other states.

Many of the introductions listed here have not been recorded in the literature and were graciously supplied by Mr. Alvin Seale, Professor Albert W. C. T. Herre, Professor Carl L. Hubbs, Mr. W. J. Phillips, Mr. A. Cecil Harrison, and Mr. J. Lyell Clarke. Certainly there have been a great many instances in which this poeciliid was taken from one place to another for purposes of mosquito control of which no record was made, and consequently the lists given above are incomplete.

The natural range of *Gambusia affinis* has already been mentioned (page 10). Two other species, *G. nobilis* and *G. geyseri* are native to Texas. Still other members of the genus are native to the Gulf Drainage of Mexico and Central America, to both Coastal Drainages of Panama, and to the islands of Cuba, Hispaniola, Jamaica, and the Caymans and Bahamas.

The accompanying map (Fig. 14) shows the natural range of *Gambusia* and the localities into which *G.*

affinis has been introduced throughout the world. Because of the large scale of miles, the areas of introduction as shown by the map are exaggerated in some instances. The facts at hand prompt the writer to agree with the late David Starr Jordan who stated that *Gambusia affinis* enjoys a wider distribution than any other freshwater fish.

The first, and most extensive, investigation on the use of fish in the control of mosquitoes undertaken by the United States Government was inaugurated as a public health measure in July 1915 (Smith 1916). The U. S. Bureau of Fisheries and the U. S. Bureau of Entomology, with the assistance of the U. S. Public Health Service, found it necessary to study the comparative efficiency of different fishes in controlling mosquito larvae and to ascertain how the effectiveness of the more suitable species was affected by the various conditions of depths of water, vegetation, and debris. An economic circular (Radcliffe 1915) was issued giving an account of the common fishes known to be of value in the control of mosquito larvae. The majority of this work was carried on in the "malaria belt" of the southeastern United States, and the mosquitofish was found to be more effective than any other species.

Hildebrand (1919, 1921, 1925), in an attempt to gain information relative to the actual value of *Gambusia* as eradicators of mosquito larvae and pupae under a wide range of conditions, made a thorough investigation over a period of four summers in the vicinity of Camp Hancock at Augusta, Georgia. The information secured from these experiments indicated that the mosquitofish had effected a reduction in the number of mosquito larvae and pupae amounting to 57.8 per cent in the anopheline and 80.8 per cent in the culicine populations. His data showed that reduction was materially greater in the number of pupae (74.2 per cent in anophelines and 85.1 per cent in culicines) than in the numbers of larvae (57.6 per cent in anophelines and 80.7 per cent in culicines). However, it was pointed out that the numbers of small larvae were reduced to a greater extent than were the large ones. In conclusion, Hildebrand listed the following reasons why *Gambusia affinis* is especially suited to antimosquito work:

"(a) It seeks its food at the surface; (b) it is very prolific; (c) it gives birth to well-developed young, therefore requiring no special environment for depositing and hatching the eggs; (d) it lives and thrives under a large variety of conditions and frequents areas especially suitable for the support of mosquito larvae; (e) it usually lives and multiplies in ponds stocked with predacious fishes, providing it has very shallow water for refuge."

H. H. Howard (1920a, 1920b, 1922) obtained effective control over mosquito production by the use of *Gambusia* in a rural area of Hinds County, Mississippi. Howard's results from the use of fish as a control measure as compared with those achieved by oiling showed that: (1) fish control is less expensive—the cost is limited to the labor of capture and trans-

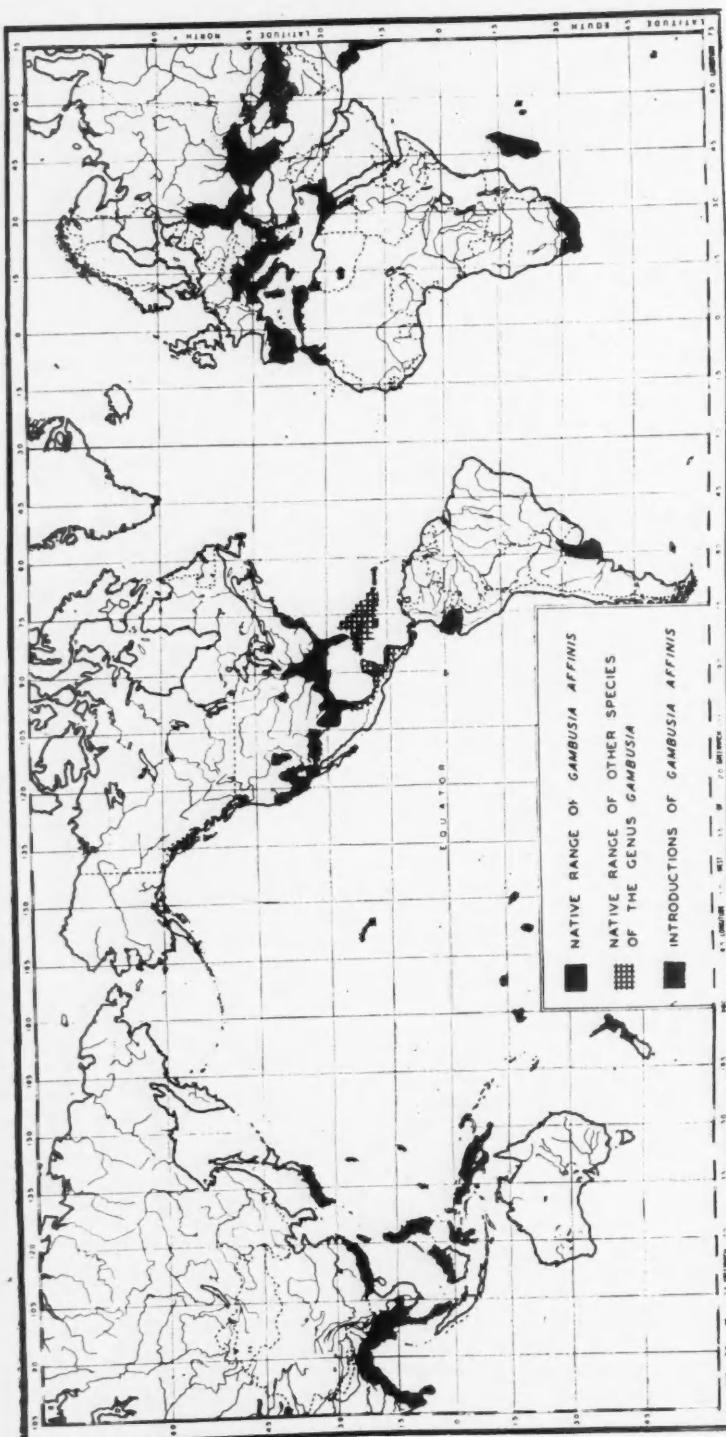


FIG. 14. World map showing the native range of *Gambusia affinis* and other members of the genus and the different parts of the world into which the mosquitofish has been introduced as a mosquito-control measure.

portation; (2) fish control is less liable to accidents—the effectiveness of the oil film is quickly destroyed by winds, rains, and wave action, which have practically no effect on the efficacy of the mosquitofish as a control agent; and (3) fish control is more enduring in its results—one introduction of fish will suffice for a long period of time whereas oiling as a control measure is transient in its results and to be effective must be regularly and continuously practiced. In addition, Howard pointed out that the incidence of malaria during 1919 was only about 65 per cent of that of the preceding year.

Moore (1922), in an attempt to find a species indigenous to the North Atlantic States which would prove as valuable as *Gambusia* in mosquito control, made observations on the larvivorous capabilities of 29 species of fishes belonging to 9 families. He found 9 species which deserved special mention, and of these the pumpkinseed proved itself to be the best of the lot but not nearly so effective as the mosquitofish. Attempts were made to acclimatize *Gambusia* to the region of Philadelphia each of the three years of the investigation. All of the attempts failed, however, as had the earlier attempts of Seal, presumably because of the severity of the winters. Moore, realizing that annual reintroductions of mosquitofish into the many mosquito-breeding habitats in the region of Philadelphia would be impractical, even though such a practice would be of value, stated:

“... The planting of a permanent self-perpetuating stock only would suffice. Up to the present such stocking in the latitude of Philadelphia has failed. Just why *Gambusia* will not live over winter is not clear. The winter of 1920-21 was no more sever than those to which the top minnow is frequently exposed within its natural range, yet in four ponds in which they thrived during the preceding summer, not a single one appears to have survived. In an attempt to solve this second problem, it is proposed to search the northern border of the fish's range for a cold-resistant race or to attempt the isolation of such a race by selection and possibly by hybridization.”

So far as is known to the writer, such a hardy race has not been established in the eastern United States.

Perhaps the most thorough treatise on the use of fish in controlling mosquitoes is the booklet “The Use of Fish for Mosquito Control” published by the International Health Board of the Rockefeller Foundation (1924). In addition to a discussion of the use of *Gambusia* in various parts of the United States, and also Spain and Italy, that paper includes a comprehensive treatment of the use of various kinds of fishes in all parts of the world where malaria is a menace. Recently, Gerberich (1946) annotated a bibliography of papers relating to the control of mosquitoes by the use of various kinds of fish.

Following the introduction of *Gambusia* into foreign lands, most workers in those countries have found that the fish are highly efficient in the control of mosquitoes. Near Rovigno, Italy, Sella (1929) found, in a single test pond where *Gambusia* was the sole agent

for the control of anopheline production, that the fish were more effective during August than at any other time during the summer. The degree of control afforded by the mosquitofish for two successive years follows:

	1927	1928
May	2%	2%
June	16%	12%
July	70%	54%
August	87%	83%

Sella attributed the better control late in the summer to the tremendous increase in the population of *Gambusia* during June and July.

Conversely, Valli (1928) found that *Gambusia* were only 20 per cent effective in mosquito control. He attributed the poor control to the abundance of aquatic vegetation which contributed considerably to the hiding of the mosquito larvae.

In the region of Kavieng, New Ireland, Holland (1933) introduced *Gambusia affinis* into all known breeding places of *Anopheles* over a coastal area 72 miles long, containing 32 villages and 3,001 natives (960 children). No mosquitofish were placed in the remainder of the subdivision which included 35 villages and 3,438 natives (890 children). The spleen rate in the “treated” area fell from 24.6 in August 1930, to 4.2 in July 1932 whereas in the same time interval the spleen rate in the “untreated” part of the subdivision fell from 47.1 to 28.8. Moreover, in the treated area there was an excess of births over deaths for the first time on record.

Sokolov (1936), studying the value of *Gambusia* in mosquito control in rice fields in Turkestan, found that the extermination of *Anopheles* larvae by the mosquitofish amounted to about 90 per cent of the number of larvae in the control ponds. The results described by Sokolov clearly indicate the role of *Gambusia* as an agent in successfully applying a biological method of combating anopheline production in rice fields.

Sieault & Roule (1935), working in Morocco, found that if mosquitofish were present in numbers in excess of 20 per square meter they prevented all mosquito multiplication during the warm weather. However, in the spring and fall, when the temperature fell below 5° C., the fish sought the upper water and allowed the mosquitoes to breed freely in the shallow water.

De Buen (1940) found that, through their voracity, *Gambusia*, although not 100 per cent effective, kept the transmitters of malaria at a minimum in Spain.

Within recent years there has been considerable research on the habits of *Gambusia* and its ability to control mosquito production in different parts of the United States. In the Southwest the mosquitofish has been introduced into many places and has been found to be quite valuable in antimosquito work. Particularly in California this poeciliid has multiplied in large numbers and has become acclimatized in many parts of the state. In Utah (Rees 1934, 1945), where

difficulty was encountered in holding the fish over winter, natural warm springs in the vicinity served as reservoirs for keeping the fish until the following summer. Recently, the mosquitofish survived in certain cold water ponds during mild winters.

In the vicinity of Chicago, Illinois, the Des Plaines Valley Mosquito Abatement District has been using *Gambusia* as an integral part of their campaign against "pest" mosquitoes since 1933. Each spring the various pools, marshes, and lakes of the region are checked for mosquitofish and if the population of the previous year has failed to survive the winter a new introduction is made. The acclimatization of the topminnow to northern Illinois and southern Michigan has been discussed previously. During late August 1938, the writer made examinations of 24 ponds southwest of Chicago which were suitable for fish during at least part of the summer. Early in the summer 14 of the ponds had been stocked with *Gambusia* and the remaining 10 had not. In those ponds in which no fish had been placed as a control measure, mosquito larvae and pupae, both anopheline and culicine, were taken in large numbers. In the 14 ponds where mosquitofish were present, only a few larvae were collected from 9 of the ponds and none could be found in the other 5.

The possible effectiveness of the mosquitofish in controlling the production of anopheline mosquitoes in southern Michigan was tested during 1943 in 4 small artificial ponds at the State Fish Hatchery at Drayton Plains. The ponds (Fig. 15) were built during the winter of 1936-37 and each was provided with a separate inlet and outlet. All of the ponds were similar in size, 100 by 50 feet, with maximum depths of about 4 feet. The water was shallow at the end where the inlet was located and became gradually deeper toward the outlet end. The bottoms of the ponds were mud to which some sand and gravel had previously been added. A heavy growth of *Chara* covered the pond floor. Some cattails and smartweeds grew in the shallower parts of each pond and some *Najas* was present in the deeper parts. Early in the summer the ponds were drained and all fish removed. Then the ponds were refilled and the aquatic vegetation was allowed to grow rampant for the rest of the summer so that natural ponds in the area might be simulated.

Each of the 4 ponds was inspected for mosquito larvae at approximately weekly intervals during the entire summer. One dip with a quart-size dipper, taken every 10 feet of shoreline assured an adequate sampling of the population of mosquito larvae in each pond. The contents of each dipperful was carefully scrutinized for aquatic stages in the life cycle of the mosquito and all specimens taken were grouped as larvae or pupae.

On July 23, 2 of the ponds (Ponds 7 and 8) were each stocked with 100 mosquitofish and the other 2 (Ponds 9 and 10) were used as controls. One of the control ponds (Pond 9) became polluted during the latter part of August and could no longer be used in



FIG. 15. Experimental minnow ponds at the Drayton Plains State Fish Hatchery. These ponds were used in testing the effectiveness of mosquitofish in controlling anopheline mosquitoes. (Courtesy of Michigan Institute for Fisheries Research.)

the experiment. The cause of pollution was not found. The water was milky and had a foul odor and the animal life in the pond was at a minimum for the rest of the summer.

The experiment was repeated the following year using 6 ponds instead of 4. Accordingly, mosquitofish were stocked in 3 ponds (Ponds 6, 7, and 8) on July 14 and the other 3 ponds (Ponds 5, 9, and 10) served as controls. At the height of the anopheline breeding season (September 1), after the weekly collections had been made, 125 mosquitofish were seined out of Pond 7 and placed in one of the control ponds (Pond 9). Those fish appeared to be representative of the population in Pond 7 inasmuch as they ranged in size from newborn young to adult males and gravid females.

The mosquitofish brought about a considerable reduction in the abundance of the anopheline larvae and pupae in the hatchery ponds under observation (Tables 20 and 21; Fig. 16). The data indicate that *Gambusia* was 80.9 and 94.8 per cent effective in the control of anopheline mosquito production in 1943 and 1944 respectively. Furthermore, when the topminnows were introduced into a fish-tree pond in the middle of the mosquito breeding season the numbers of larvae and pupae decreased sharply in subsequent collections, whereas the numbers of larvae and pupae taken in the collections from the 2 remaining control ponds continued to increase for another 2 weeks. Although control was not as complete as that in ponds in which mosquitofish had been present during the entire summer, the production of *Anopheles* was greatly reduced.

In all probability the growth of aquatic vegetation in the hatchery ponds used in this experiment was not as rank as that in nearby natural waters of comparable size. However, the fact remains that *Gambusia* effected at least an 80 per cent control of anophelines breeding under experimental conditions which simulated those in nature. Also, the topminnows wintered over in the ponds and were present in

TABLE 20. Numbers of anopheline larvae and pupae collected from three ponds (the data from Pond 9 were not used) at the Drayton Plains State Fish Hatchery, 1943. Ponds 7 and 8 contained *Gambusia* and Pond 10 was used as a control.

Collection date	POND 7		POND 8		POND 10	
	larvae	pupae	larvae	pupae	larvae	pupae
July 23	21	...	9	...	8	...
July 30	33	...	12	...	62	...
August 6	46	...	7	...	107	3
August 13	55	...	18	...	164	5
August 27	19	2	19	...	155	2
September 9	9	...	8	...	103	1
September 17	2	2	30	5
September 24	3	1	3	...	37	4
October 4	12	1
October 8	8	...
Total	188	5	76	...	686	21

sufficient numbers the following spring to supply an ample stock for further distribution of the species. Thus, the permanence of the control afforded by the use of the mosquitofish, together with the very low cost of introduction and maintenance, adds considerably to the practical value of this fish in antimosquito campaigns.

SUMMARY

A total of 30,093 specimens of *Gambusia a. affinis* were collected during 3 summers from 8 ponds in northeastern and central Illinois. All of those fish were measured, sexed, and classified as mature or immature individuals. The numbers of eyed embryos in the broods carried by 2,381 gravid females were counted.

Length, frequency, size distribution, and growth rate.—Female mosquitofish attain greater lengths than males because the females continue to grow until they die whereas males grow very little after the formation of the gonopodium is completed. Consequently, the

largest specimen in any collection of *Gambusia* is usually a female.

Length frequencies and size distributions of mosquitofish in collections from Illinois depend to a great extent on the fertility of the pond and the time of

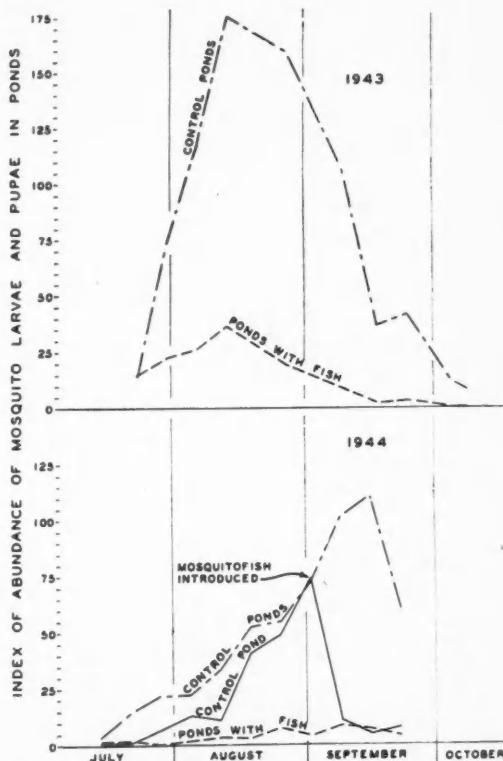


FIG. 16. Numbers of anopheline larvae and pupae collected from ponds at the Drayton Plains State Fish Hatchery, 1943 and 1944. The data represented in the graphs are given in Tables 20 and 21.

TABLE 21. Numbers of anopheline larvae and pupae collected from six ponds at the Drayton Plains State Fish Hatchery, 1944. Ponds 6, 7, and 8, contained *Gambusia*, and Ponds 5, 9, and 10, were used as control ponds. *Gambusia* were introduced into Pond 9 on September 1.

Collection date	POND 6		POND 7		POND 8		POND 9		POND 5		POND 10	
	larvae	pupae	larvae	pupae								
July 14	3	...	1	...	1	6	1
July 21	4	1	...	6	...	23	...
July 28	1	...	1	...	6	1	5	...	38	1
August 4	3	13	...	6	1	38	...
August 11	4	3	...	11	...	10	1	54	2
August 18	1	...	5	40	1	27	...	63	4
August 25	3	...	5	...	7	...	48	1	30	...	80	...
September 1	5	1	2	...	70	4	52	1	92	...
September 8	5	...	4	2	6	...	11	...	64	...	131	8
September 15	4	...	8	...	2	...	5	...	69	...	151	2
September 22	4	...	3	...	1	...	8	...	40	4	68	8
September 29	3	...	2	...	2	...	8	...	33	1	65	7
October 6	2	...	3	2	...	20	...	44	4
Total	29	...	43	3	25	...	224	7	362	8	853	37

summer at which the collection is made. Samples taken during May contained only relatively large fish that had survived the preceding winter whereas young-of-the-year mosquitofish were invariably present in collections made later in the summer. Considerable differences in the growth rate of *Gambusia*, with consequent differences in size range, occurred in different ponds. Females, both parent and offspring, of the fast growing population in the fertile Argonne Woods Pond reached greater lengths and exhibited greater size ranges than did those in the less fertile Sanitary District Lake. In Parr's Pond, where the fertility was higher and the growth rate faster than in either of the Chicago ponds, too few parent females were taken to allow comparison but the female offspring attained a maximum length of 49 mm. during their first summer of life as compared with similar measurements of 44 mm. and 34 mm. respectively for female offspring from the Argonne Woods Pond and the Sanitary District Lake.

The largest mature male mosquitofish taken from the Sanitary District Lake was 36 mm. long as compared with maximum sizes of mature males of 32 mm. from the Argonne Woods Pond and 31 mm. from Parr's Pond. However, in all collections from those 3 ponds there were only 4 specimens more than 31 mm. long.

Accordingly, the maximum size of the female mosquitofish in Illinois depends largely on the fertility of the pond in which she lives whereas that of the male seldom exceeds 31 mm.

Size and age at first maturity.—Inasmuch as mosquitofish are promiscuous in their breeding habits the minimum size at which the young reach maturity is rather easily defined by the lengths of the smallest mature males and gravid females in adequate collections. The determination of the maximum size at which males become mature is obviated by the fact that growth practically ceases with the complete formation of the gonopodium. On the other hand, it is considerably more difficult to ascertain the maximum size at which females become gravid for the first time. Parent females, which were present in the ponds near Chicago at the beginning of the summer and had obviously survived the previous winter, became gravid for the first time early in their second summer of life. The maximum size for such females in collections from the Argonne Woods Pond was 45 mm. whereas those from the Sanitary District Lake were 31 mm. long. Those fish were 8-10 months old.

Some of the offspring of the parent fish became gravid during their first summer. The minimum sizes for such gravid offspring in the collections from the Argonne Woods Pond, the Sanitary District Lake, and Parr's Pond were 31 mm., 26 mm., and 24 mm. respectively. Those offspring were approximately 6 weeks old when they first became gravid.

Male mosquitofish reach maturity at smaller sizes and at slightly earlier ages than the females. The parent fish in the Argonne Woods Pond, the Sanitary District Lake, and Parr's Pond exhibited maximum

lengths of 36, 32, and 31 mm. respectively whereas the offspring became mature at minimum lengths of 21, 21, and 19 mm. respectively.

Length of the reproductive period.—The female mosquitofish present in a wild population at the beginning of the summer in Illinois are virgin. Those fish become gravid for the first time at an age of 8-10 months and, after liberating a single series of broods, disappear from the population. Their reproductive period extends over 10-15 weeks depending on the number of broods liberated. Female offspring of the first and possibly the second brood liberated by the parent fish usually become gravid at an age of 4-6 weeks. These offspring, having been fertilized, give birth to a single series of broods and then die. Such offspring have a reproductive period 4-10 weeks long depending on the number of broods they bear.

The length of time males of *Gambusia* remain potent is not definitely known. It has been pointed out by other workers that the formation of sperm is a continuous process once maturity has been reached. Functionally, there is little need for male mosquitofish to retain their potency over long periods of time inasmuch as one fertilization of a female is adequate for the production of the single series of broods to which she gives birth.

Gestation period.—The gestation period for *Gambusia* ranged from 21 to 28 days in 14 such periods observed in the laboratory. The average for the 14 periods was 23.9 days.

Number of broods born per season.—The number of broods liberated by a female mosquitofish during a season in Illinois ponds depends on the age at which maturity is reached and, to a lesser extent, on the fertility of the pond in which she lives. Parent females, having reached maturity at an age of 8-10 months, give birth to a series of 3-4 broods whereas offspring that become mature when 4-6 weeks old, liberate only 1-2 broods. In Parr's Pond, where the population was newly established and the fertility of the pond was very high, the parent females gave birth to 4 and possibly 5 broods and the offspring liberated at least 3 broods of young.

Relationship between the length of the mother and the size of the brood.—In general, the average number of young per brood increases with the length of the mother fish. The physical limitation of the size of the mother fish is not the sole determining factor in the relationship between the gravid female and the number of young per brood. The rate of increase in the size of the brood is not constant with the rate of increase in the length of the mother fish but rather decreases as the length of the mother increases. Some metabolic factor, coincident with the approaching end of the reproductive season, apparently controls the rate of increase in the number of young per brood.

Decrease in the size of broods with increasing age.—The decrease in fecundity with increasing age holds for both parent fish and offspring. The average number of young per brood carried by females in Illinois

ponds showed that even though the actual length of the mother increased there was a marked decrease in the size of the broods in subsequent collections.

Senility.—Poeciliids, unlike many other fishes, have a definite period of sterile senility following reproduction. Senility among *Gambusia* is not a function of age alone. Females that began to bear young when only 4-6 weeks old were physiologically more senile at an age of 4 months than were females that did not begin to reproduce until they were 8-10 months old. Furthermore, the older fish reproduced over a longer period than did those which reached maturity at an early age.

Length of life under natural conditions.—In Illinois no mosquitofish was found that had survived 2 winters and entered the third summer of life (2 annuli on the scales). In general, mosquitofish die the same summer in which they reach maturity. The maximum life span is that of females which do not become mature until their second summer. Those fish liberate their series of broods and die at an age of about 15 months.

Sex ratio and differential death rate.—Male and female mosquitofish are born in approximately equal numbers. As the fish grow older there is a gradual shift in the sex ratio in favor of the females. The females are hardier and longer-lived than the males. Unequal sex ratios in collections of *Gambusia* are not to be attributed to a differential birth rate but rather to the relatively longer life and hardiness of the females.

Acclimatization to northern Illinois and to Michigan.—*Gambusia* native to southern Illinois have become acclimatized to natural waters in northern Illinois and have withstood the rigors of the climate in that region for more than 20 years. Some of the hardy stock from the Chicago area were introduced into outdoor ponds in southeastern Michigan in 1941 and have survived 6 winters there.

Mosquito control.—Experiments extending over 2 summers in Michigan showed that *Gambusia* was 80.9 and 94.8 per cent effective in the control of anopheline mosquito production in ponds where natural conditions were simulated. When introduced into a fish-free pond at the height of the mosquito breeding season, mosquitofish effected about an 80 per cent reduction in the anopheline reproduction within a week.

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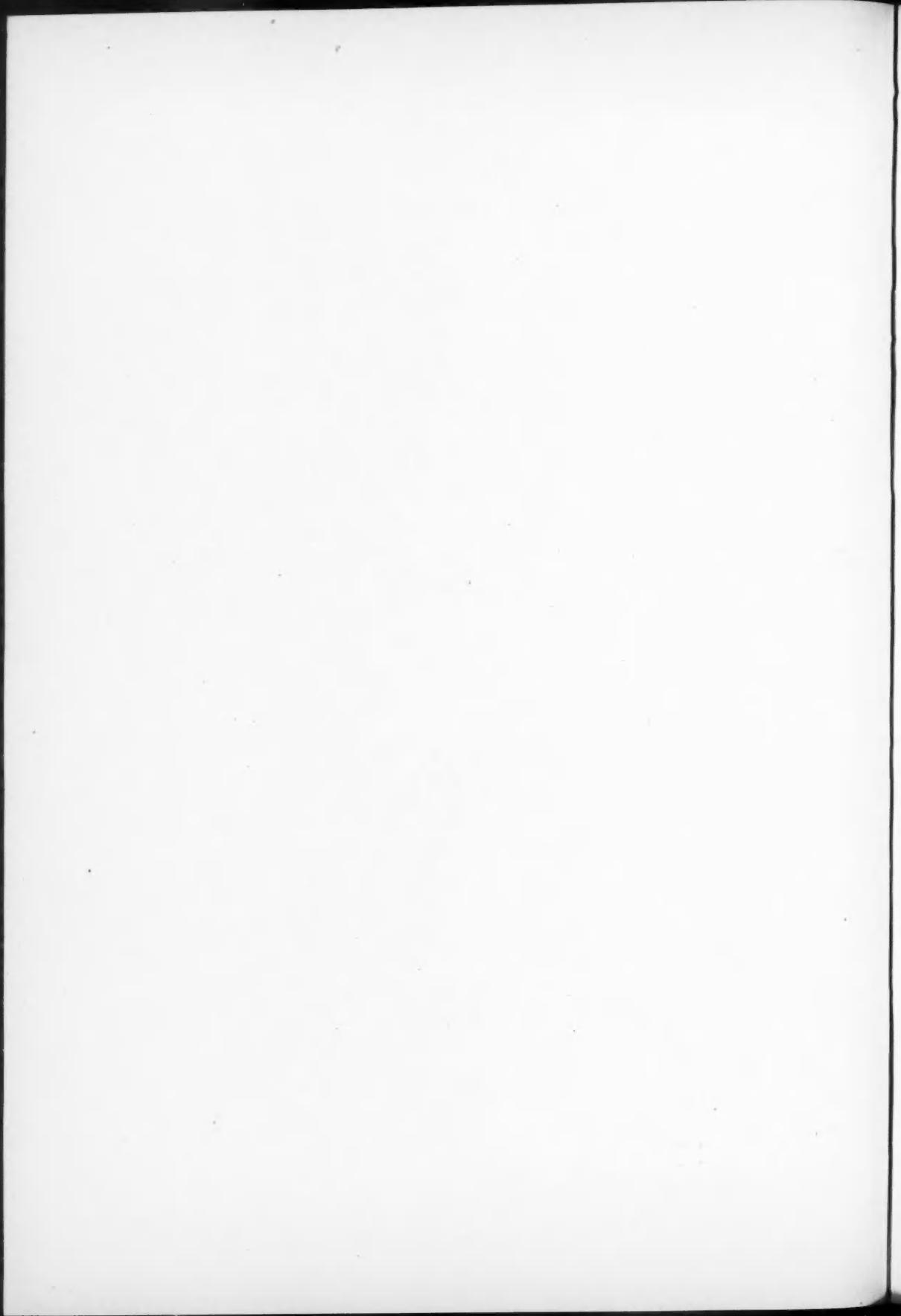
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BIONOMIC STUDIES ON THE BEET LEAFHOPPER

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BIONOMIC STUDIES ON THE BEET LEAFHOPPER

INTRODUCTION

The beet leafhopper (*Eutettix tenellus* (Baker)) (Order Hemiptera, Suborder Homoptera, Family Cicadellidae) the only known vector of the virus of curly top disease, has been the most important pest affecting sugar beets in the Western States and has also been outstandingly important in limiting the production of beans, tomatoes, melons, and other garden crops, and many ornamental plants. Since the agent of transmission of the disease was announced by Ball in 1906, extensive investigation of the problem has included studies of the life history, biology, and host plants of the insect in relation to weather and climatic conditions in the more important areas of distribution as well as epidemiological research and the breeding of disease-resistant varieties of plants.

Bionomic studies of the beet leafhopper have been especially difficult because of the rapid fusion or overlapping of successive generations, long migrations, and more or less local shifting movements, and the difficulty of determining and measuring effects of the more significant variables in the environmental complex. Such studies have also been complicated by seasonal variation in the time of germination and maturation of different wild host plants and their sequence, abundance, and distribution. Owing to the involved ecological relations and the difficulty of studying certain phases of the effects of weather and climatic factors by methods other than analytical, experiments were conducted to serve as a practical basis for field studies and as an aid in interpreting problems of development, activities, abundance, and distribution of the insect.

The investigations on which this paper is based were conducted as a part-time project from 1932 to 1937, inclusive, at Twin Falls, Idaho, in cooperation with the Bureau of Plant Industry, Soils, and Agricultural Engineering and the Idaho Agricultural Experiment Station. The study was initiated by P. N. Annand, to whom appreciation is expressed for direct supervision during earlier phases of the work. The writers are also indebted to W. C. Cook for his interest in the studies and many helpful suggestions given during the progress of the work and preparation of the manuscript. Other assistance given in various ways by R. A. Fulton, C. F. Henderson, J. C. Chamberlin, A. O. Larson, D. E. Fox, and M. F. Bowen is gratefully acknowledged.

PREVIOUS STUDIES

The beet leafhopper was announced to be the vector of curly top disease of sugar beets by Ball (4) in

1906, who also recorded early observations on its life history and wild host plants (5, 6). Ball (6) concluded that only one generation of the beet leafhopper occurs annually, but Stahl (87) and later investigators (14, 29, 41, 49) have correctly reported the occurrence of two or more generations annually in the more important areas of distribution. A detailed account of the life history of the leafhopper in California, supplemented by studies of the life cycle in observation cages was given by Severin (82, 83).

Carter (14) extensively analyzed the beet leafhopper problem with special reference to the ecology of the insect in southern Idaho. Annand *et al.* (2) discussed movements of the leafhopper from different desert breeding areas in southern Idaho and emphasized the importance of extensive and thorough surveys in the fall and spring as a closer check on the distribution, abundance, and development of the insect relative to economic conditions determined by the magnitude and time of the spring movement to the cultivated areas.

Seasonal development, natural enemies, and host-plant relations of the insect in northern Utah were discussed by Knowlton (49, 50). Hills (41) studied seasonal development of the leafhopper and effects of weather conditions on its abundance in south-central Washington and northeastern Oregon. Studies of types of vegetation in the San Joaquin Valley of California and effects of overgrazing and intermittent farming on the distribution and abundance of seasonal weed hosts of the beet leafhopper were reported by Piemeisel & Lawson (72) and Lawson & Piemeisel (53). Fox (24) described the occurrence of the beet leafhopper and associated insect species on secondary plant successions in southern Idaho with reference to studies by Piemeisel (71) of the changes in weedy plant cover that follow the abandonment of cleared sagebrush lands. Romney (76) discussed the breeding areas and economic distribution of the beet leafhopper in New Mexico, southern Colorado, and western Texas, and methods of its control on beets grown for seed in Arizona and New Mexico (77). The life history, bionomics, and control of the leafhopper were discussed by Cook (16, 17).

METHODS

Groups of eggs for the different experiments were obtained by placing mature females on sugar-beet plants in small cages designed to fit snugly around the petioles. After 12 hours the cages were removed and portions of the plant in which the eggs were embedded were marked with ink. Toward the end of embryonic development, cages were replaced on the plants to confine the young nymphs. The same

cages were used to confine single adult females in experiments on oviposition. Transfer of the females to fresh plants was accomplished by shaking the cage lightly until the insects would come to rest on the side of the container, when it was slipped off an exposed plant and onto a fresh one. One of these cages, which was designed and used by Charles F. Henderson in related studies, is shown in Figure 1A.

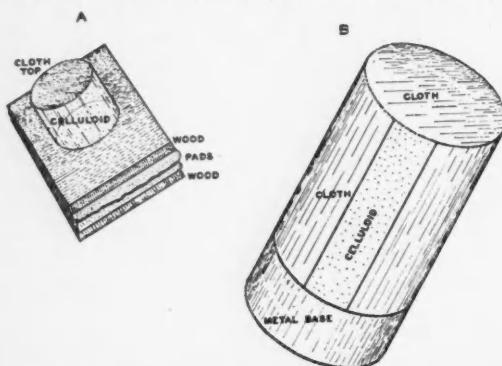


FIG. 1. Cages used to confine the beet leafhopper for studies under controlled conditions: A, for studies of egg and preovipositional stages; B, for studies of the nymphal stage.

Cages used in studies of nymphal development (Fig. 1B) were made of thin, fine-meshed cloth over a supporting frame of celluloid attached to a metal band. These were placed over the beet plant and pressed into a layer of clean sand covering the surface of the soil so that both leaves and petioles of the plant were available for feeding. Both types of cages were designed to provide a maximum circulation of air and thus to minimize any effect of evaporation on temperature and humidity conditions within the cages.

The cabinets in which the cages were placed have been described elsewhere (2a). The temperature of the cabinets was controlled within approximately $\pm 0.2^\circ$ F. and the relative humidity within ± 1 percent. Continuous records of conditions within the cabinets were obtained with hygrothermographs, and temperature and humidity within the cages were checked with a thermometer and small dewpoint tube after methods described by Smith (86). Temperature changes and various periods of exposure at different temperatures were obtained by transferring the insects from one chamber to another at definite intervals. The relation of the temperature of the plants to air temperatures and the amount of temperature lag following transfers from higher to lower temperatures and vice versa were checked by means of small thermocouples inserted carefully in the plant tissue to insure placement of the junctures below the cuticle. The influence of temperature lag was apparently negligible, since differences of only a few degrees over periods of 2 to 6 minutes were detected, and these effects were evidently rather closely compensating in alternate transfers. Possible cooling effects of evapo-

ration were minimized by watering the plants sparingly from below in saucers.

Egg deposition, hatching, attainment of the adult stage, and first egg deposition occurred under the same experimental conditions as were maintained for the corresponding embryonic, nymphal, and preovipositional periods. The eggs were deposited during a 12-hour period, 9 a.m. to 9 p.m. or vice versa, and hatching was recorded daily at 9 a.m. and 9 p.m. in timing the embryonic period. The nymphs hatched or reached the adult stage over a 24-hour period and were observed daily at 9 p.m. in measuring the time required for the corresponding nymphal and preovipositional stages.

In studies of oviposition the eggs were counted as follows: The plant petioles were labeled with small string marking tags and immersed in a saturated solution of chloral hydrate, which was heated almost to the boiling point and then permitted to cool. The same solution was used repeatedly. This treatment rendered the plant tissue transparent and usually stained the eggs a light green. The eggs were counted by examining the plant material in a clear, concentrated solution of chloral hydrate under a binocular microscope. A photomicrograph of eggs in the plant tissue after treatment is shown in Figure 2.

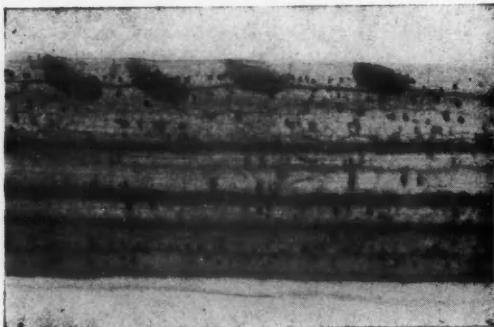


FIG. 2. Photomicrograph of eggs of the beet leafhopper in a beet plant after treatment with chloral hydrate.

Population samples on the desert plots were taken at random according to the following procedure: The observation plot was divided into smaller sections, 15 feet square, by placing numbered stakes at the corners. Beans, previously numbered to correspond with the numbers of the smaller plots, were drawn from a carton to determine the general distribution of the samples over the larger plot. From another series of beans numbered 1 to 15, two numbers were drawn to determine the coordinates of the position in the smaller plot where the sample was to be taken.

Samples were taken at the predetermined positions by throwing down a cage held within a circular die mounted on the end of a pitchfork handle. The sampling cage was a cylinder of transparent celluloid that was capped with fine-meshed cloth and mounted on a cylindrical sheet-metal base for insertion within

the cutting die. To remove the samples the die was forced into the soil to a depth of about 5 inches by means of a rotary motion. During the winter, and at other times when the soil was moist, the contained sample could be removed undisturbed by lifting the die. At other times, to avoid disturbing the contained soil and roots of the plants, it was necessary to remove the soil from one side of the position with a trowel and to insert and hold a metal disk beneath the sample while it was removed from the die.

The samples were placed in a delivery truck and brought to the laboratory for examination under a strong light. When necessary the samples were watered in a shallow metal tray when placed in the truck. Nymphs and adults were removed from the sampling eages by means of a suction tube and were recorded according to the nymphal instars and sex and seasonal color forms of the adult. After examination the samples were incubated for a week at 92° F. to permit hatching of the eggs and were then reexamined to determine the number of eggs that had been present on the date of sampling. From 20 to 50 samples were taken at weekly intervals during the summer and 40 to 50 samples were taken every 2 weeks during the winter. These methods and the apparatus were developed and used in cooperation with D. E. Fox, who was studying the seasonal abundance of the beet leafhopper relative to that of some of its more important predators and other associated arthropods (36a).

BRIEF OUTLINE OF THE LIFE HISTORY

In southern Idaho the beet leafhopper overwinters in the adult stage chiefly in sagebrush areas and abandoned dry-farming sections where there are mustards or other suitable host plants. The insects are active and feed during the winter whenever the temperatures permit. The overwintering females are fertilized in the fall, and most of the males die during the winter. Preoviposition development is completed during the winter, and egg laying usually begins early in March. Leafhoppers of the first, or spring, generation develop in the same areas chiefly on flixweed (*Descurainia sophia* (Lam.) Webb) and green tansy-mustard (*Descurainia pinnata* subsp. *filipes* (Gray) Detling) and usually reach the adult stage by the end of May. The influx of adults of the spring generation to the cultivated areas begins at this time, and the movement reaches a peak 1 to 3 weeks later, depending chiefly on temperature conditions affecting maturation of the insects in the contributing breeding areas.

In this season temperatures permit rapid ovarian development, and the insects begin egg laying on arrival in the beet fields or within a few days thereafter. One generation or one and a part of another mature in beet fields during the remainder of the season.

During the spring movement of leafhoppers to cultivated areas, weedy sections in the deserts and in abandoned and cultivated farms are also populated.

Russian-thistle serves as the most important breeding host during the summer. Maturation of the second generation, or the third or overwintering generation of the season, occurs on Russian-thistle late in September and in October, when cooler weather produces the darker markings that characterize the overwintering adults. Another dispersal occurs as the third generation matures, and the leafhoppers again appear in the overwintering and spring breeding areas, where they enter the winter on fall-germinated mustards or less favorable plants that serve as hold-over food hosts.

STUDIES OF THE EGG AND NYMPHAL STAGES

DEVELOPMENT

Effects of constant temperatures

The incubation periods at different constant temperatures are given in Table 1. The time-temperature relationships for duration of the egg stage and the relative effect of different temperatures on the rate of development are illustrated in Figure 3.

The data show that the rate of embryonic development is rather closely proportional to the temperature between 65° and 93° F. and is well represented within

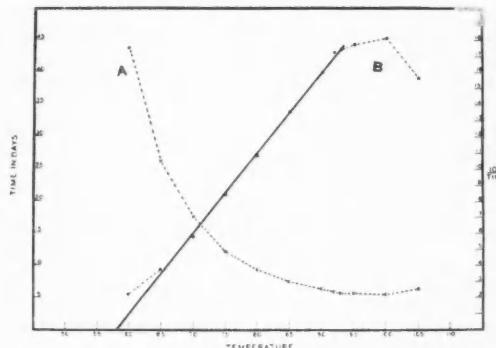


FIG. 3. Development of the beet leafhopper at different constant temperatures: A, Duration of the egg stage; B, rate of embryonic development.

TABLE 1. Duration of the egg stage of the beet leafhopper at different constant temperatures and 50% relative humidity.

Temperature (°F.)	Eggs observed	Time required	Index rate of development	Thermal increment
				Day-degrees
105	121	6.37 ± 0.06	15.70	299
100	547	5.51 ± .02	18.15	231
95	1,258	5.63 ± .01	17.76	208
93	552	5.74 ± .03	17.42	201
92	532	5.77 ± .04	17.33	196
90	603	6.27 ± .02	15.95	200
85	1,274	7.41 ± .01	13.50	200
80	511	9.23 ± .03	10.83	202
75	791	11.93 ± .02	8.38	202
70	270	17.40 ± .06	5.75	208
65	280	26.35 ± .06	3.80	183
60	32	43.84 ± .30	2.28	85

this range of temperature by a linear regression line. The values for the rate of development are indices equal to 100 divided by the time. The equation for the regression line, when fitted by the method of least squares, is $100/D = -29.1171 + 0.5014 T$, in which $100/D$ is the rate of development and T is the temperature in Fahrenheit degrees.

In Figure 3 the straight line is extended to the zero line of the time scale, and this intersection at 58.07° F. indicates the threshold temperature where development theoretically ceases as the temperature is lowered. Actually the minimum temperature seems to be between 55° and 50° , but much of the value of the regression line data at higher temperatures depends on the point selected for zero development, as the thermal increments approach a constant value only when temperatures are measured from this level.

This was shown by Krogh (51) from the linear relation, $v = kx$ between temperature (x) and the rate of development (v). Since the rate of development is expressed as the reciprocal of the time ($v = 1/y$), the equation may be written, $1/y = kx$, or $1/k = x/y$, in which the product of time and temperature is a constant. In the same way the equation $100/D = -29.1171 + 0.5014 T$ can be expressed as $100/0.5014 = D(T - 29.1171/0.5014)$ or $D(T - 58.07) = 199$ day-degrees. The product of the calculated duration (D) and temperature counted above 58.07° F., or $T - 58.07$, gives the same number of day-degrees for any point on the regression curve.

The thermal increments for the observed duration of the egg stage show that approximately the same number of day-degrees are required for embryonic development at different constant temperatures from about 65° to 95° F. (Table 1). At higher and lower temperatures the observed values deviate in opposite directions from the regression line (Fig. 3) and the thermal increments (Table 1) show corresponding departures from the calculated thermal constant of 199 day-degrees.

The duration of the nymphal stage of development at different constant temperatures is given in Table 2. Time-temperature relations for the nymphal period and the relative effect of different temperatures on the rate of development are illustrated in Figure 4.

The regression lines in Figure 4 have the equations $100/D = -13.2290 + 0.2255 T$, and $100/D = -12.8095 + 0.2180 T$, for males and females, respectively. In the time-temperature form the equations become $D(T - 58.67) = 443$ day-degrees and $D(T - 58.76) = 459$ day-degrees, respectively. Males complete the nymphal stage in about 3.5 percent less time than is required by the females. The regression equations for averages of the values for both sexes are $100/D = -12.9897 + 0.2214 T$, and $D(T - 58.67) = 452$ day-degrees.

Approximately the same number of day-degrees (Table 2) are required for nymphal development at different constant temperatures from 65° to 93° F. The observed values for rates of development at

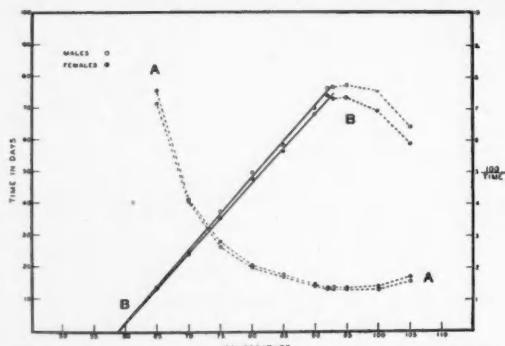


FIG. 4. Development of the beet leafhopper at different constant temperatures: A, Duration of the nymphal stage; B, rates of the nymphal development.

higher and lower temperatures deviate in opposite directions from the regression line (Fig. 4) and the thermal increments (Table 2) show corresponding departures from the calculated thermal constant of 452 day-degrees.

The thermal requirements for development from egg to adult, therefore, include 452 day-degrees above 58.67° F. for the nymphal stage plus 199 day-degrees above 58.07° for the egg stage, or a total of about 650 day-degrees. In round numbers these may be considered as 450, 200, and 650 day-degrees, respectively.

Embryonic periods of 6.05 ± 0.02 , 12.49 ± 0.04 , 17.09 ± 0.13 and 25.99 ± 0.11 days at 90° , 75° , 70° , and 65° F., respectively, for eggs of light colored adults collected during the summer do not show any marked differences from those just discussed and probably indicate that the thermal increments for development do not differ in progeny of the different seasonal color forms. Duration of the developmental stages of progeny of overwintered females collected from several of the Western States was also about the same when compared at the same time at 90° and 50% relative humidity. The embryonic periods were 6.31 ± 0.02 , 6.41 ± 0.02 , 6.41 ± 0.03 , and 6.27 ± 0.02 days for progeny of adults from California, Colorado, Arizona, and Idaho, respectively, and the nymphal stage in the same order required 14.27 ± 0.11 , 14.58 ± 0.12 , 14.63 ± 0.11 , and 14.50 ± 0.07 days.

Nymphs of the beet leafhopper were observed to molt five times when confined singly in small cages, and measurements of nymphs in different stages of development also indicate that there are five instars which may be reliably distinguished by the width of the head capsule. The weight prior to the final molt increases with lower temperatures and the nymphs are definitely larger, but frequency distributions of the head width of nymphs reared at 100° and 75° F. did not merge or indicate that there would be any overlapping of the measurements of successive stages developed at different temperatures. Averages of the head width in successive instars at the two temperatures are shown in Table 3. The ratio of increase

TABLE 2. Time required for the development of nymphs of the beet leafhopper at different constant temperatures and 50% relative humidity.

Temperature (°F.)	INSECTS REARED		TIME REQUIRED		INDEX RATE OF DEVELOPMENT		THERMAL INCREMENT	
	Males	Females	Males	Females	Males	Females	Males	Females
105.....	Number	Number	Days	Days			Day-degrees	Day-degrees
105.....	210	193	15.59 ± 0.17	17.03 ± 0.19	6.41	5.87	722	788
100.....	288	256	13.23 ± .11	14.44 ± .11	7.56	6.93	547	596
95.....	245	241	12.96 ± .10	13.63 ± .11	7.72	7.34	471	494
93.....	325	316	13.02 ± .10	13.74 ± .11	7.68	7.28	447	471
92.....	307	285	13.14 ± .08	13.52 ± .08	7.61	7.40	438	450
90.....	209	214	14.32 ± .10	14.67 ± .10	6.98	6.82	449	458
85.....	280	244	17.10 ± .11	17.68 ± .12	5.85	5.66	450	464
80.....	164	149	20.07 ± .12	20.89 ± .15	4.98	4.79	428	444
75.....	228	235	26.69 ± .19	28.28 ± .16	3.75	3.54	436	460
70.....	171	165	40.44 ± .24	41.17 ± .25	2.47	2.43	458	463
65.....	53	33	70.91 ± .53	75.42 ± .81	1.41	1.33	449	471

in head width seems fairly constant in accordance with the principle of Dyar (21) as is shown by comparison of the observed means with the corresponding values calculated by least squares. Estimated by least squares, the average increase in width of the head capsule is 26 to 27 percent in successive instars. Other studies (Harries & Henderson 37) show that the head width of successive instars increases about 25 percent but only about 5.5 percent from the fifth instar to the adult.

TABLE 3. Average width, in millimeters, of the head capsule of successive instars of the beet leafhopper reared at 100° and 75° F. and 50% relative humidity.

Instar	100° F.		75° F.	
	Observed	Calculated	Observed	Calculated
1.....	0.326 ± 0.001	0.331	0.328 ± 0.004	0.333
2.....	.422 ± .005	.420	.428 ± .005	.420
3.....	.544 ± .006	.532	.532 ± .005	.529
4.....	.678 ± .006	.673	.667 ± .005	.667
5.....	.838 ± .005	.853	.836 ± .009	.841

Average durations of the different nymphal instars at 90° F. and 50% relative humidity are shown in Table 4. As noted by Carter (14), the first and last instars are of longer duration than are the intermediate stadia.

TABLE 4. Average duration of the different stadia of the beet leafhopper in laboratory rearings at 90° F. and 50% relative humidity.

Stadium	Nymphs observed	Average duration	
		Number	Days
1.....	45	3.38 ± 0.10	
2.....	44	2.29 ± .11	
3.....	43	2.39 ± .11	
4.....	41	3.07 ± .14	
5.....	38	3.76 ± .20	

The average weight approximately doubles in successive instars, and at the end of the nymphal stage it is more than twenty times that at hatching. The daily increase in weight at 90° F. and 50% relative humidity is shown in Table 5. These data, illustrated in Figure 5A, define a typical sigmoid curve of growth in which the daily increment or rate of growth increases with increased weight of the insect during the major portion of the stage and then declines as the

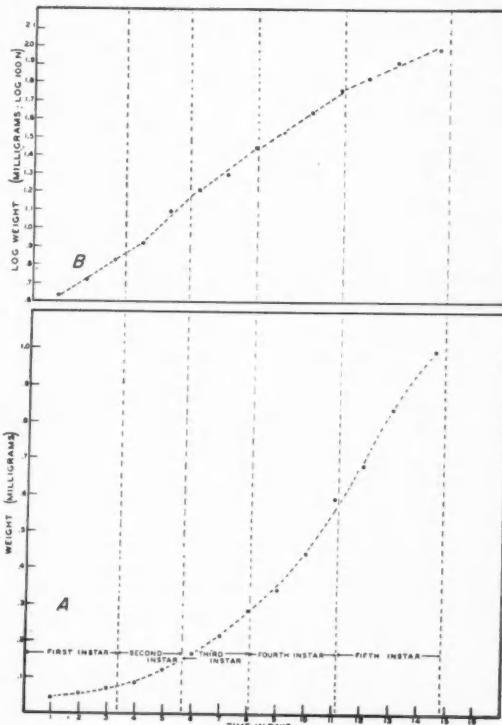


FIG. 5. Nymphs of the beet leafhopper during development at 90° F. and 50 percent relative humidity: A, Average daily weight; B, logarithms of weights.

adult stage is approached. When logarithms of the weights are plotted against time to illustrate the ratio of increase in weight during the nymphal stage, the growth curve appears to consist of 5 linear segments of different slope, indicating that growth occurs at different rates in successive periods which evidently correspond to the different instars (Fig. 5B). Weight of the nymphal and adult stages was observed and recorded by weighing lots of 50 nymphs or 10 adults at about the same hour each day. Similar S-shaped curves were shown by Brindley (10) for larval growth of the Mediterranean flour moth (*Ephestia kuhniella* Zell.) and the confused flour beetle (*Tribolium confusum* Duv.) at a constant temperature.

TABLE 5. Daily increase in weight of the beet leafhopper, nymph to adult stage, at 90° F. and 50% relative humidity.

Age	Individuals weighed	Average weight	Growth ratio
Days	Number	Mg.	
1	300	0.043	
2	350	.053	1.23
3	400	.067	1.26
4	400	.084	1.25
5	400	.126	1.50
6	500	.168	1.33
7	450	.198	1.18
8	400	.279	1.41
9	500	.335	1.20
10	550	.448	1.34
11	650	.592	1.32
12	500	.680	1.15
13	550	.835	1.23
14.5 ¹	260	.994	1.19

¹In adult stage.

Temperature coefficients

Although it seems doubtful that there is any fundamental basis for relating simple equations to physiological processes that may be the resultant of many reactions, both chemical and physical, such expressions are often of considerable interest because of their general descriptive value. Effects of temperature show many contrasts in the descriptive value and theoretical significance of different analytical expressions. In general, many of the data define curves of increasing slope in which the rate of activity appears to be some type of exponential function of temperature (20, 26, 33, 34, 40, 46, 51, 65, 74, 75, 84, 92) while other results indicate that the rate of activity is closely proportional to temperature as would be described by a straight line (9, 34, 51, 56, 68, 78, 85, 93).

In Figures 6 and 7 data on duration of the egg and nymphal stages are related to different expressions advanced by Krogh (51), van't Hoff (43), Arrhenius (3), and Belehrádek (7). The data are plotted according to graphs of the equations in linear form, and the extent of agreement is shown by the

approach of the values to lines of best fit calculated by the method of least squares.

A quantitative expression of the idea that completion of a definite period of development requires a definite amount of heat expressed in temperature summations was presented by Sanderson & Pears (78) in 1913. In 1914 Krogh (52) and Blunck (9) described the same relationship, which is often referred to as Blunck's or, more commonly, as Krogh's law. According to this principle, as expressed by Krogh's formula ($V_{t10} = V_t + K_{10}$), the increase in rate of development is proportional to the rise in temperature. Close agreement of data on the egg and nymphal stages with the Krogh equation was discussed previously as a basis for summing temperatures, but is illustrated in Figures 6 and 7 for comparison with that of other analytical expressions.

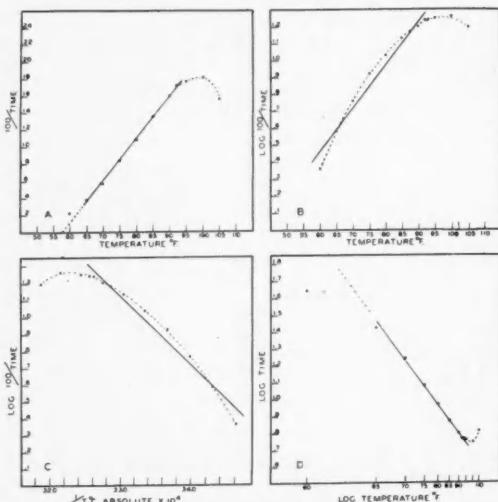


FIG. 6. Data on duration of egg stage of the beet leafhopper at different temperatures plotted with reference to the analytical expressions of A, Krogh; B, van't Hoff; C, Arrhenius; and D, Belehrádek.

According to the equation of van't Hoff, the rate of activity is a simple exponential function of temperature in which the ratio of increase in rate is constant and is expressed as Q_{10} , or in terms of a difference of 10 Centigrade degrees in temperature. The logarithm of rate is therefore proportional to the temperature in the linear form of the equation. When the data are plotted in this way in Figures 6, B, and 7, B, for the egg and nymphal stages, respectively, the distribution of points does not approach linearity for any appreciable range in temperature and from the curvilinear trend of the values relative to the calculated lines it is evident that Q_{10} decreases progressively with higher temperatures and cannot therefore be a constant for the processes.

The equation of Arrhenius associating rate of chemical reactions with temperature has also been widely used in describing the effect of temperature

on biological processes. In the linear form of the equation the logarithm of rate is proportional to the reciprocal of the absolute temperature. Figures 6, C, and 7, C, show that the data are not in agreement with the formula for any appreciable range in temperature.

Belehrádek (7) has related the effect of temperature on certain biological processes to an influence of protoplasmic viscosity on rates of diffusion at different temperatures, by the formula $y = a/x^b$ where y is time, x is Centigrade temperature, and a and b are constants. In this equation the logarithm of time is proportional to the logarithm of temperature in the linear form, $\log y = \log a - b \log x$. In some instances as was pointed out by Belehrádek, agreement of data with the equation sometimes depends on a correction made by counting temperatures above the biological minimum for the process instead of zero Centigrade. In such cases where the temperature scale is so adjusted, the rate of activity ($1/y$) becomes more proportional to temperature as the value of the constant b approaches one, and when b equals one the equation becomes identical with that of Krogh (Belehrádek 7). In fitting the present data there was no substantial agreement with the equation until temperatures were counted from levels considerably above zero Centigrade. The best agreement seems to be obtained when b approaches a value of one in counting temperatures above 58° F. or near the theoretical developmental zeros indicated by intersection of the regression lines with the temperature axis in Figures 6A, and 7A. The close agreement shown in Figures 6D, and 7 D may therefore be only incidental, because the rate of development is closely proportional to temperature, but Belehrádek (7) suggested that the Krogh expression is a special case of the more general equation relating physiological processes to temperature.

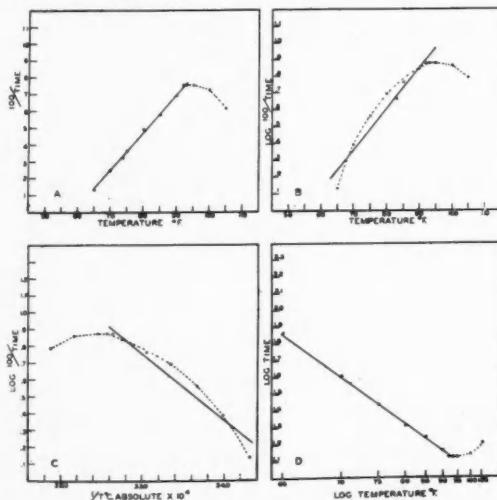


FIG. 7. Data on duration of the nymphal stage of the beet leafhopper at different temperatures plotted with reference to the analytical expressions of A, Krogh; V, van't Hoff; C, Arrhenius; and D, Belehrádek.

Effects of alternating temperatures

Duration of the embryonic period under alternating high and lower temperatures is given in Table 6, together with the theoretical time that should be required on the basis of data shown in Table 1 for full-time development at each of the two temperatures. The time theoretically necessary for development in alternate exposures to two different temperatures was computed as follows: Since the egg stage was completed in 5.51 days at 100° F. and in 6.27 days at 90° , the sum of the percentages, $12/24 \times 100/5.51$ plus $12/24 \times 100/6.27$, for alternate exposures of 12 hours daily at each temperature represent 17.05 percent of the developmental time per day and the theoretical time necessary for development is, therefore, $100/17.05$, or 5.86 days.

Comparison of the observed and calculated values shows that the egg stage was generally completed in less time than that theoretically necessary, or that development was relatively faster under alternating temperatures than at thermally equivalent constant temperatures. The amount of acceleration averages about 5 percent under the different conditions, but appears to be influenced by the temperatures involved, since a marked trend is shown toward greater acceleration as the range between the higher and lower temperatures increased.

Data on duration of the nymphal stage under alternating temperatures are shown in Table 7, together with the time theoretically required for development on the basis of results obtained at constant temperatures (Table 2), computed as previously described for similar data on the egg stage. Nymphal development was also completed in less than the expected time, with an average acceleration of about 8 percent apparently resulting from the alternate changes in temperature.

Reference to Figures 3 and 4 or Tables 1 and 2 shows that temperatures between 95° and 100° F. are relatively non-effective and should not be included in the summation. The inhibitory effect of still higher temperatures must also be taken into account when appreciable accumulations occur above 100° , and in this case the summation may be roughly adjusted by subtracting the number of day-degrees above 100 from the effective total summed between 58° and 95° . This adjustment for the higher temperatures, in the example of data on the effects of constant and alternating temperatures in Tables 1, 2, 6, and 7, brings the values much nearer the respective constants of 200 and 452 day-degrees for the egg and nymphal stages as is shown by the following summations:

°F.	EGG STAGE		NYMPHAL STAGE	
	Day-degrees	°F.	Day-degrees	°F.
105	203	105	511	
100	203	100	503	
100-90	200	100-95	442	
100-85	196	100-80	440	
100-65	188	100-75	451	
100-40	187			

TABLE 6. Duration of the egg stage of the beet leafhopper at alternating constant temperatures, together with the calculated time based on constant-temperature values. Relative humidity was about 95% at 40° F. and 50% at all other temperatures.

12 HOURS DAILY AT EACH TEMPERATURE

Alternating Temperatures (°F.)	Eggs observed	Time required	Calculated time	Deviation	Thermal increment
	Number	Days	Days	Percent	Day-degrees
100 and 90.....	320	5.82 ± 0.02	5.86	-.68	215
100 and 85.....	294	6.13 ± .02	6.32	-3.01	211
100 and 65.....	97	8.58 ± .05	9.11	-5.82	210
100 and 40.....	90	10.13 ± .02	11.02	-8.08	212
95 and 90.....	212	5.70 ± .02	5.93	-3.88	196
95 and 85.....	514	6.14 ± .02	6.40	-4.06	196
92 and 80.....	249	6.69 ± .03	7.10	-5.77	187
92 and 65.....	436	8.93 ± .02	9.47	-5.70	182
93 and 40.....	849	10.67 ± .01	11.48	-7.06	186
90 and 85.....	356	6.54 ± .02	6.79	-3.68	192
90 and 70.....	427	8.74 ± .02	9.22	-5.21	192
90 and 65.....	429	9.53 ± .03	10.13	-5.92	185
90 and 60.....	267	10.12 ± .03	10.97	-7.75	171
85 and 75.....	247	9.21 ± .03	9.14	+.77	202
85 and 70.....	113	10.01 ± .03	10.39	-3.66	194
85 and 65.....	234	10.93 ± .04	11.56	-5.45	185
80 and 75.....	353	9.88 ± .02	10.41	-5.09	192

8 HOURS DAILY AT HIGH TEMPERATURE AND 16 HOURS DAILY AT LOW TEMPERATURE

80 and 60.....	231	19.06 ± .07	19.49	-2.21	164
90 and 60.....	52	13.42 ± .07	14.63	-8.27	160
90 and 50.....	150	16.15 ± .05	18.81	-14.14	172

TABLE 7. Duration of the nymphal stage of the beet leafhopper at different alternating constant temperatures when held for 12 hours daily at each temperature. Relative humidity was about 95% at 40° and 50% at all other temperatures.

Temperature (°F.)	INSECTS KEARED		TIME REQUIRED		CALCULATED TIME		DEVIATION		THERMAL INCREMENT	
	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
	Number	Number	Days	Days	Days	Days	Percent	Percent	Day- degrees	Day- degrees
100 and 95.....	85	91	11.71 ± 0.14	12.64 ± 0.17	13.26	14.25	-11.69	-11.30	455	490
100 and 85.....	101	113	13.50 ± .15	14.56 ± .14	14.91	15.90	-9.46	-8.43	457	491
100 and 70.....	71	56	18.35 ± .20	19.52 ± .23	19.92	21.37	-7.88	-8.66	483	512
95 and 90.....	73	81	12.73 ± .15	13.52 ± .17	13.79	14.35	-7.69	-5.78	430	456
95 and 85.....	82	74	13.28 ± .10	14.04 ± .14	14.96	15.65	-11.23	-10.29	416	439
95 and 70.....	90	93	17.72 ± .19	18.01 ± .18	19.61	20.49	-9.64	-12.10	422	428
95 and 40.....	88	90	25.12 ± .20	25.77 ± .22	25.92	27.26	-3.69	-5.47	456	467
93 and 80.....	178	194	15.71 ± .14	16.44 ± .14	15.80	16.58	-0.57	-.84	437	456
92 and 75.....	165	152	16.80 ± .11	17.21 ± .11	17.61	18.28	-4.60	-5.85	417	426
93 and 65.....	161	131	19.99 ± .12	20.85 ± .18	22.03	23.20	-9.26	-10.13	407	422

Effects of time of exposure to different temperatures

In the effects of alternating temperatures on embryonic development, a definite trend toward greater acceleration is shown as the range between the higher and lower temperatures increased (Table 6). Cook (15) has shown in studies of cutworm larvae that the effect of alternating temperatures is determined by the time of exposure to different temperatures as well as by the temperatures involved. In alternately

subjecting the cutworm larvae to different temperatures for various periods of exposure, Cook found that the amount of acceleration increased with greater differences in temperature within a range of 8° to 32° C. and with shorter daily exposures of 8 and 4 hours at the higher temperature.

Data in Table 8 indicate that the percentage of embryonic development of the present species also increases with decrease in time of exposure from 20 to 8 hours daily at the higher temperature. Further

studies of this phase of the effect of temperature on embryonic development of the beet leafhopper (35) have shown that in exposures of 4, 8, 12, 16, and 20 hours daily at 90° F. alternating with exposures for the remainder of each 24-hour period at 80°, 70°, 60°, or 50°, the percentage of acceleration increased with shorter exposures at the higher temperature and with greater differences between the higher and lower temperatures. Inasmuch as the daily period of higher temperatures tends to shorten with increase in the daily range of temperature occurring in the spring and fall, both factors of temperature and time of exposure would apparently act to the advantage of the species in permitting more development than would otherwise occur in the cooler seasons of the year.

TABLE 8. Duration of the egg stage of the beet leafhopper under varying periods of exposure to high and low temperatures and 50% relative humidity at both temperatures.

HOURS OF EXPOSURE AT		Eggs observed	TIME		Deviation
90° F.	65° F.		Required	Calculated	
24	0	Number	Days	Days	Percent
		603	6.27 ± 0.02
20	4	395	7.39 ± .03	7.18	+2.92
16	8	170	8.25 ± .03	8.40	-1.79
12	12	229	9.67 ± .03	10.13	-4.54
8	16	149	12.11 ± .05	12.74	-4.95
4	20	54	16.44 ± .12	17.17	-4.25
0	24	280	26.35 ± .06

In other studies of differential effects of temperature on development of the beet leafhopper (36) a single change from a higher to a lower temperature resulted in completion of the egg and nymphal stages in a shorter time than could be expected. This effect evidently was not due to a direct stimulus, since a reverse change between the same two temperatures usually resulted in an increase in the time required rather than a decrease. Apparently there are at least two different growth phases within the egg and nymphal stages that are unequally affected by different temperatures, and these time differences indicate that the rate of development at a lower temperature is relatively less in the earlier part of the embryonic and nymphal periods and greater in the later portions, in comparison with the rates at a higher temperature, than is indicated by the average rates based on the time required for completion of the entire stages at each temperature. Therefore, the time necessary for development under alternating or variable temperatures cannot be computed accurately from durations of the entire stages at different constant temperatures.

The relatively more rapid development at lower temperatures in the later nymphal stages apparently would enable more of the larger nymphs, which occur abundantly late in the fall, to complete development than would otherwise reach the adult stage in which the insect passes the winter.

Effects of varying temperatures

Except at temperature extremes, the egg and nymphal stages have been shown to require the accumulation of approximately an equal number of effective day-degrees for development at any constant temperature, but the total effect of normally varying temperatures cannot be measured accurately on this basis because of the factor of acceleration shown in comparisons of the relative effect of constant and alternating temperatures. The direct summing of effective temperatures in day-degrees must therefore be recognized as a method of approximation, since effects of differences in temperatures involved, in time of exposure to different temperatures, and in phases of development completed at different temperatures are too complex to be taken completely into account.

Summations recorded with a thermograph under varying temperatures in a greenhouse insectary, during periods represented by the average duration of the egg and nymphal stages in groups of insects of the same age, are in fairly good agreement with the specified thermal constants. The area on the thermograph chart under the recorded line between 58° and 95° F. was measured with a planimeter and computed in day-degrees. During these experiments the daily temperatures were generally fluctuating between 55° and 90° and occasionally between 50° and 100°. Results of successive tests, for egg and nymphal stages as compared with the respective constants of 199 and 453 day-degrees, are shown as follows:

EGG STAGE		NYMPHAL STAGE	
Insects reared per test	Day-degrees	Insects reared per test	Day-degrees
860	198	184	413
174	191	236	434
153	219	181	411
		244	455
		118	405

The weighted average for tests on the egg stage falls very close to the thermal constant of 199 day-degrees, and that for the nymphal stage shows an acceleration of about 6 percent, which is a fairly common deviation in previous results obtained under alternating temperatures.

Effects of seasonal temperatures

During 1934 and 1935 studies of seasonal development were carried on by sampling representative plots in the desert breeding areas. In the spring of 1934 the plot was located near Wendell, Idaho, in a fairly uniform stand of flixweed. When drying of the mustard or spring host plants occurred, sampling was continued on another plot located in an adjacent stand of Russian-thistle, *Salsola kali* var. *tenuifolia* Tausch (Fig. 8). Hygrothermograph records were taken under an instrument shelter placed on the ground with the heat sensitive element elevated about 5 inches above the surface of the soil. Recordings of temperatures between 58° and 95° F. were measured with a planimeter and computed in day-degrees for comparison with seasonal development of the leafhopper. Soil-surface temperatures and records of



FIG. 8. Second plot at Wendell, Idaho, showing stand of Russian-thistle and subdivision by stakes placed at the corners of the smaller plots.

precipitation were also obtained during the progress of the studies.

The spring of 1934 was exceptionally early, and the spring generation of leafhoppers matured much earlier than normally. Egg laying of the overwintered insects began early in March. Nymphs of the spring generation had begun to appear when sampling was started on April 18. The light-colored adults of the spring generation began to appear about April 28, and the beginning of the migration or influx of these adults to the cultivated areas was noted about the same time. Standard-shade air temperatures recorded at the laboratory in Twin Falls, Idaho, during maturation of the spring generation in 1934 totaled only about one-half the necessary day-degrees between 58° and 95° F., but summation of soil-surface temperatures were about equal to the required amount.

During the spring, temperatures recorded in the shade are evidently inadequate as a measure of the actual environment, since the day-degree summations up to the date of the spring movement rarely total more than one-half of the necessary amount for development through the egg and nymphal stages. In this season of the year the insects seem especially subject to soil-surface temperatures, which generally range much higher than air temperatures and are more than sufficient to account for the seasonal development observed. The host plants are usually very short during the egg and early nymphal stages in the spring, and on cold days, and sometimes when air temperatures are near or below the minimum for development, nymphs are observed to obtain much higher temperatures exposed to full sunlight on the plants or barren areas of soil.

Adults of the spring generation were most numerous about May 15. Eggs and nymphs of the

second generation were most abundant about May 20 and June 5, respectively, and between these dates the accumulation of effective temperatures at 5 inches above the ground was approximately equal to the required sum of 200 day-degrees for duration of the egg stage. Adults of the second generation were approaching maximum abundance by the middle of July and the required sum of effective temperatures, or about 450 day-degrees, was accumulated between June 6 and July 11.

There was considerable overlapping of the second and third generations, but the presence of a third generation of the season was clearly indicated by the abundance of eggs and small nymphs in the last half of July following maturation of the second generation adults. Nymphs of the third generation were most abundant about the end of July, following accumulation of the necessary increment of 200 day-degrees for the egg stage during the last half of the month, and reached the adult stage late in August and early in September, after approximately the required sum of 450 day-degrees for nymphal development had been recorded during August.

During maturation of the second generation from about May 15 to July 15, and the third generation between July 15 and August 31, there was a slight excess of recorded temperatures that would about account for preoviposition development of the second generation, but otherwise the progress of seasonal development seemed to check at least as closely with the recorded thermal increments as the peaks of abundance could be determined in estimating the average duration of the stages.

Temperatures accumulated after the end of August, when the third generation matured, were sufficient for a partial fourth generation. However, owing to the

early spring and the effects of extreme drought and higher-than-normal temperatures during the summer, the Russian-thistle was maturing and drying prematurely during September, and the appearance of eggs and nymphs of a fourth generation on the plot was apparently prevented by unfavorable condition of the host plants.

The population studies were continued during 1935 on a plot near Berger, Idaho, where there was a fairly uniform stand of spring host plants, mostly flixweed, and a sufficient admixture of Russian-thistle for plant succession to carry the leafhoppers through the season. In this year spring development of the leafhopper was about 2 weeks later than usual, but temperatures during the remainder of the summer were about normal.

Oviposition of the overwintered adults in 1935 began during the first part of May, and the eggs were most numerous about May 20. Nymphs began to appear about May 10 and were most abundant around the first of June. Adults of the spring generation were first noted about June 5, when the movement began, and reached a maximum toward the middle of June. As in the preceding year, the first nymphs of the spring generation matured well before the necessary accumulation of effective air temperatures had been recorded, but records of soil-surface temperatures exceeded the required summation during the same period.

Insufficient data were obtained on the occurrence of eggs during the remainder of the season, but nymphs of the second generation began to appear early in July and were most abundant toward the end of the month. Maturation of the second generation was delayed by lower than normal temperature accumulations during the first half of July. Increments of temperature recorded between peaks of abundance of nymphs of the first and second generations on about June 1 and August 1, respectively, were near the estimated amount necessary for a generation. Apparently the peak of abundance of the second-generation adults came about September 1, or after the accumulation of approximately 450 day-degrees during August.

Nymphs of the third generation appeared during the latter half of August and were reaching maximum abundance about the middle of September, or when about 200 day-degrees necessary for development in the egg stage had been accumulated following the peak of abundance of the second generation adults. Adults of the third generation began appearing in late September and were becoming most abundant in October during colder weather. Only about two-thirds of the necessary temperature accumulation for maturation of nymphs of the last generation was recorded after September 1. Many larger nymphs were present with the advent of colder weather, but many or most of these seemed to complete development with the aid of higher soil-surface temperatures.

In 1935 population samples were also taken in beet fields at weekly intervals during the season. The

movement of leafhoppers of the spring generation to beet fields began about June 5, when the first insects were reaching the adult stage in the desert. The number of adults reaching the beet fields increased slowly at first and then more rapidly to about June 25 and the increase coincided generally with the progress of maturation of the spring generation in the desert breeding areas. Ovarian dissections and cage tests indicated that the leafhoppers began ovipositing within 2 or 3 days after arriving in the beet fields. Nymphs were abundant in the latter part of July. Adults of this generation produced in the beet fields reached a peak of abundance in late August and early September, as the corresponding second generation of the season was maturing on wild host plants in the desert areas. Only one complete generation of leafhoppers developed in beet fields during the season, and few if any adults of another generation appeared before harvest of the beets in October.

Considerably greater accumulations of effective temperatures for development are obtained in the actual environment than are recorded between 58° F. and 95° by the standard shade thermograph (5 feet above the ground). Also, temperatures at different levels above the ground are apparently not closely enough correlated to establish an accurate basis for summing standard-shade temperatures for this purpose. Comparisons of standard-shade temperatures, accumulated above 40°, 45°, 50°, and 55° on the thermograph charts, indicate, however, that such temperatures should be summed above levels of 45° to 50° to correspond most closely with those near the ground or between 58° and 95° in the actual environment of the insect.

Summations of standard shade temperatures between 50° and 90° F. at Twin Falls, Idaho, from thermograph records available since 1926 have given the following seasonal accumulations:

Year	Day-degrees	Year	Day-degrees
1926	2,700	1932	2,400
1927	2,300	1933	2,600
1928	2,600	1934	3,300
1929	2,500	1935	2,600
1930	2,700	1936	2,700
1931	2,900	Mean	2,664

This method seems to underestimate the actual temperatures during the spring, according to dates of the maturation and movement of adults of the first generation to the cultivated areas, but tends to overestimate the effective seasonal totals as compared with those taken near the ground in 1934 and 1935 during sampling studies of seasonal development on the desert plots.

The 11-year average of 2,664 day-degrees is about equal to the estimated sum required for three generations, including preovipositional development of the first and second generations, and is nearly the same as that shown above for 1935, when maturation of the third generation seemed fairly complete. The seasonal accumulations thus seem to check fairly well with field observations that there are 3 generations

annually in southern Idaho, the last generation sometimes maturing late in the season and perhaps limited by a deficiency of annual temperatures.

The beet leafhopper was reared through several successive generations in the green house and apparently will continue to reproduce indefinitely under favorable temperature conditions. The number of generations occurring annually appears, therefore, to be determined by the seasonal temperature accumulations in the different areas of distribution. As many as 15 to 16 developmental cycles, through the egg, nymphal, and preovipositional stages, would be possible annually under experimental conditions of about 95° F., but since the relatively long oviposition period permits some of the earlier progeny to mature before the last eggs are deposited, the minimum would seem to be about 8 and the average number of cycles about 12. Since considerably more than half of the eggs are deposited during the first half of the oviposition period, an average of about 12 cycles seems to be a conservative estimate of the number of complete generations possible annually under the most favorable experimental conditions for rapid development. Apparently, temperatures in the hotter areas of the Southwestern States would produce 6 to 8 complete generations annually, although their identity is lost later in the season because of overlapping or fusion of the succeeding generations.

Effect of Temperature on the Time of Migration

The first influx of beet leafhoppers to the cultivated area has generally been considered as marking the date of migration. The major part of the movement usually occurs considerably later, and its progress coincides generally with maturation of the spring generation. The movement increases slowly at first and then more rapidly, and reaches a peak usually 1 to 3 weeks later, depending on weather conditions, chiefly temperature affecting the rate of maturation of the insects in the contributing breeding areas.

From the time egg laying begins in the spring the leafhopper requires an accumulation of about 650 day-degrees between 58° and 95° F. to reach the adult stage. The major portion of the movement cannot occur much before this total is obtained. The thermal constant of 650 day-degrees is based on mean durations of the egg and nymphal stages in groups of the same age, and when development occurs at different constant temperatures. Because of the accelerative effect of varying temperatures, and difference in the minimum and average times for development under the same conditions, the beginning of the movement could be expected somewhat in advance of the date indicated by the specified thermal increment necessary for maturation of the spring generation. Frequency distributions of durations of the stages under the same conditions show that the minimum is uniformly about 15% less than the mean, regardless of temperature. Estimating the acceleration at from 5 to 10% on the basis of data on development at alternating temperatures, and under varying temperatures in the insectary, the total reduction of 20 to

25% would correspond to from 75 to 80% of the normal temperature increment, or about 490 to 520 day-degrees.

Comparison of the observed migration dates and soil-surface temperatures at Twin Falls for the years 1927-34, for which complete records are available, shows an average accumulation of about 513 day-degrees above 58° and below 95° F., after March 1 and preceding the beginning of the movements (Table 9). In comparison with standard shade temperatures measured in the same way for the years 1927-36, the migrations always began considerably before the amount necessary for development to the adult stage had been recorded. This illustrates the inadequacy of standard shade temperatures as a measure of the actual environment of the insect especially during the spring when the host plants are very short. In this connection, Carter (14: 12; Tables 3, 4) has shown that soil-surface temperatures generally exceed those of standard shade throughout the year and that the maximum temperatures at 6 inches above the ground were consistently higher during the winter and spring months than those of the soil surface or at any other level. Also, during the spring on bright calm days when the heat of reflection is greater and less rapidly dissipated, the insects may be observed feeding, or resting on exposed areas of the soil, under favorable to optimum temperatures when standard shade temperatures are well below the minimum for development or even for physical activity.

Evidently it is such increments of favorable temperatures that enable the insect to complete ovarian development during the winter months and thus the time when egg laying begins in the spring may be determined. When day-degrees of effective soil-surface temperatures from different dates back to November 1 of the preceding year are included in the summation, the correlation between the observed migration dates and the recorded temperatures is not improved (Table 9).

Although the thermal requirements for development can be determined rather accurately under controlled conditions, the intraenvironmental differences in temperature make it difficult to correlate closely seasonal development with the available thermograph records. On the basis of correlation with the actual environmental temperatures, standard shade temperatures summed above 45° F. are also compared in Table 9 with the observed dates of the initial migrations. This level seems near the appropriate base for summing standard shade temperatures since sums above 50° average about two-thirds of the necessary accumulation for maturation of the spring generation, and above 40° the required increment is exceeded by about the same amount. By including the day-degrees accumulated during the period December through February, the average deviation of the temperature summation from the 10-year mean is reduced from 65 to 48 day-degrees, indicating only a slight improvement in the estimate of the mean. Inclusion of the data for November does not improve this estimate.

By taking 670 day-degrees above 45° F. (Table 9)

TABLE 9. Summations of standard shade temperatures above 45° F. and of soil-surface temperatures above 58° and below 95° from different dates to the time of the spring migration of the beet leafhopper to the cultivated areas.

STANDARD SHADE TEMPERATURES ABOVE 45° F.

Year	Migration date	Day-degrees of Temperature Counted from				
		March 1	February 1	January 1	December 1 ¹	November 1 ¹
1927	June 13	703	707	709	717	778
1928	May 14	461	462	464	472	555
1929	June 9	705	705	705	706	754
1930	May 24	580	621	622	678	732
1931	do	648	650	650	650	695
1932	June 13	765	765	765	766	819
1933	June 6	639	639	644	648	710
1934	April 28	578	634	646	679	739
1935	June 5	672	676	676	677	777
1936	May 25	704	707	707	707	708
Average		646	656	659	670	727
Average deviation		65	55	54	48	48

SOIL-SURFACE TEMPERATURES ABOVE 58° AND BELOW 95° F.

1927	June 13	496	496	496	496	
1928	May 14	406	406	406	406	422
1929	June 9	690	690	690	690	696
1930	May 24	460	477	477	478	482
1931	do	631	631	631	631	653
1932	June 13	615	615	615	615	615
1933	June 6	405	405	405	405	414
1934	April 28	401	427	427	432	485
Average		513	518	518	519	538
Average deviation		99	95	95	95	100

¹ Of the previous year.

as the mean accumulation of standard-shade temperatures to the date of migration, the correspondence between the dates when this total is attained and those of the observed migrations is represented by a correlation coefficient of 0.957, adjusted for the number of cases. This agreement is illustrated in Figure 9. In the present series of observations the standard error of estimate of 4.27 days was exceeded once in the 10-year period (Fig. 9). This was in 1928, when the initial movement apparently began about 10 days before it could be expected according to the standard shade temperature records.

Despite the high correlation coefficient, the initial movement cannot be anticipated reliably on the basis of standard shade temperatures. This seems to be due largely to the effect of sunlight or differences in the vertical temperature gradient above the ground in different years. At least this seems to account for much of the wide discrepancy in 1928, since soil-surface temperatures in this year were about equal to those in 1933 and 1934 and were exceeded only 22% by those in 1927, when in standard shade temperatures the accumulation was 53 percent greater (Table 9). Carter (14, Table 7), in terms of recorded area on the thermograph charts above 60° F. to the initial movements, shows an accumulation of 22.1 square inches in soil-surface temperatures through May 14, in 1928, or a deficiency of about 11%

as compared with 24.9 square inches through June 13, in 1927.

According to the correlation coefficient, 0.957, about 92% of the variation in the time of migration may be accounted for by the thermal accumulations during the spring. Since this leaves about 8% of the

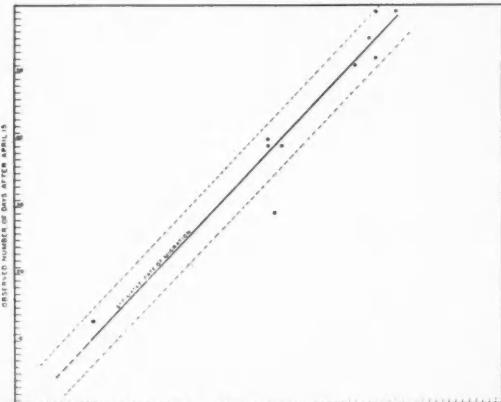


FIG. 9. Relation of estimated date determined from the standard shade temperature summations to the observed time of migration of the beet leafhopper. The dotted lines indicate the standard error of estimate of ± 4.27 days.

variation attributable to other factors, including the unreliability of standard shade temperatures as a measure of the actual environment, evidently temperature is the most important variable governing the time of migration.

Spring temperatures are especially important, because the extent of crop damage is determined by the time of occurrence as well as by the magnitude of the movement. This is because the plants are more susceptible to effects of the virus when infected in earlier stages of growth.

Temperature accumulations at Twin Falls, Idaho, for weekly intervals during the different years of study by the laboratory have been illustrated in Figure 10 in comparison with a smoothed curve approximately representing the mean summation of temperatures in successive weeks during the 12-year period. Serious curly top injury to sugar beets occurred in 1926, 1928, 1930, 1931, and 1934, when the date of movement was advanced by greater than normal accumulation of effective temperatures for development of the spring generation of leafhoppers, as is shown by the blackened area above the curve preceding the average date of migration. Temperature deficiencies from the average increments are similarly illustrated by cross-hatched areas below the curve. Good yields of sugar beets were obtained in 1927, 1929, 1932, and 1935, when the spring temperature accumulations were below normal and the initial movements occurred after the average date. Since 1935 newly developed strains of sugar beets resistant to curly top have been planted and the yields have fluctuated much less widely in different years.

Effects of humidity

Being inserted within the tissue of the plant, eggs of the beet leafhopper seem to be relatively independent of atmospheric moisture. As shown in Table

10, duration of the egg stage at different relative humidities and a constant temperature is about the same, and there is no regular order of variation that would indicate any effect of atmospheric moisture on the rate of development at 85°, 90°, and 100° F.

The data show very little difference in length of the nymphal stage for either males or females under eight different percentages of relative humidity at 92° F., but at 100° for both sexes the rate of development decreased as the relative humidity increased from 10 to 50 and to 90%. Under the distinctly unfavorable 90 percent relative humidity at 100°, where only 9 of 500 specimens completed the stage, there was a definite retardation in the rate of development. When the temperature was lowered to 70°, the stage lengthened for both males and females with an increase from 50 to 80% relative humidity. Only two conditions of relative humidity are compared in this case and the results are not very presumptive, but the time differences are statistically significant for both sexes.

In general the development of insects seems to be more rapid at the higher relative humidities. The egg stages of *Prodenia litura* (F.) (Janisch 47) and *Microbraccon hebetor* (Say) (Maercks 57); larval stages of the bean weevil (*Acanthoscelides obtectus* (Say)) (Menusan 62), *Tribolium confusum* Duv. (Holdaway 44), and *Tineola bisselliella* (Hum.) (Griswold & Crowell 28); and pupal stages of *Winthemia quadripustulata* F. (Hefley 39), *Pyrausta unicolor* (Hbn.) (Caffrey & Worthley 13), *A. obtectus* (Say) (Menusan 62), and the yellow mealworm (*Tenebrio molitor* L.) (Payne 67) were observed to require less time for development in moist than in drier air at the same temperature. Wigglesworth (94) cites other examples of this and also some cases where the rate of development was retarded at very

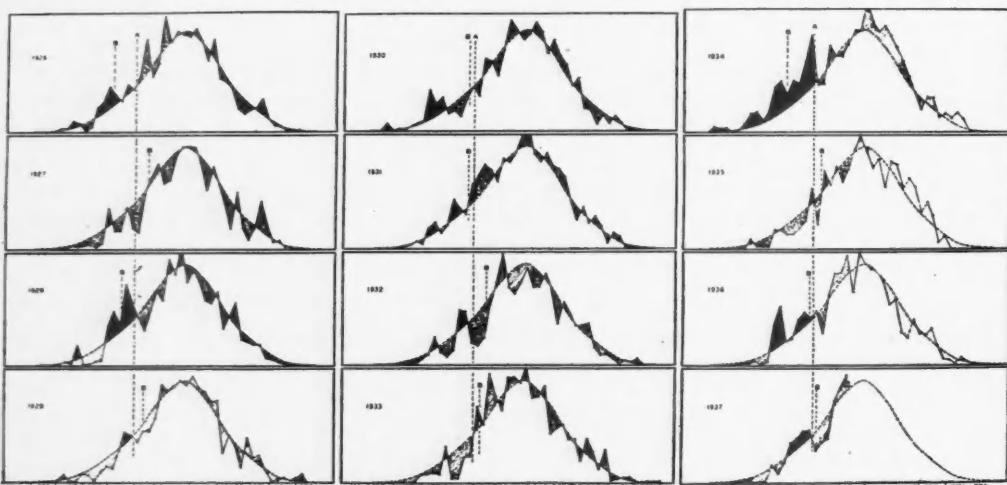


FIG. 10. Thermal summations in day-degrees of standard shade temperatures above 50° and below 90° F. for weekly intervals, 1926-1937, Twin Falls, Idaho: A, Average date of migration for the 12 years; B, date of migration for each year.

TABLE 10. Duration of the developmental stages of the beet leafhopper under different temperature and humidity conditions.

Temperature (°F.)	Relative humidity	EGG STAGE		NYMPHAL STAGE			
		Reared	Time required	Reared		Time required	
				Males	Females	Males	Females
100	10	Number	Days	Number	Days	13.98 ± 0.11	14.08 ± 0.10
	20	154	5.67 ± 0.04	179	173		
	50	547	5.51 ± .02	288	256	13.23 ± .11	14.44 ± .11
	80	204	5.85 ± .04				
	90	4	5	13.00 ± . . .	16.00 ± . . .
92	20	300	300	12.86 ± .07	13.62 ± .07
	30	250	271	13.35 ± .10	13.83 ± .09
	40	370	334	13.02 ± .07	13.68 ± .08
	50	307	285	13.14 ± .08	13.52 ± .08
	60	472	411	13.05 ± .07	13.66 ± .08
	70	170	170	13.39 ± .14	13.84 ± .15
	80	218	190	13.18 ± .09	13.85 ± .10
	90	161	130	13.10 ± .15	13.52 ± .18
	30	405	6.50 ± .03
90	50	358	6.05 ± .02
	60	264	6.37 ± .03
	80	320	5.94 ± .02
	30	105	7.56 ± .05
85	50	599	7.54 ± .05
	50	1,274	7.41 ± .01
	70	309	7.59 ± .05
70	50	171	165	40.44 ± .24	41.17 ± .25
	80	36	40	43.53 ± .54	45.05 ± .49

high humidities. Certain other insects, such as *Toxoptera graminum* (Rond.) (Headlee 38), *Heliothrips haemorrhoidalis* (Bouché) (Rivnay 75), and onion thrips (*Thrips tabaci* Lind.) (MaeGill 60), appear to be quite independent of atmospheric humidity.

Effects of light

The egg stage was slightly longer in total darkness than under about 80 foot-candles of continuous illumination, but it was found that this difference evidently resulted from an inhibiting effect of darkness on emergence from the egg after embryonic development was completed. The emergence of leafhoppers completing embryonic development during the hours of darkness would thus tend to be delayed until stimulated by sunlight and rising temperatures in the morning, and the effect of light would evidently account for much of a diurnal rhythm in which most of the eggs were observed to hatch during the early morning and daytime.

The eyes of the embryonic nymph in its later stages of development are to some extent exposed to light, inasmuch as they are readily discernible from the exterior of the plant. The poles of the egg, when in the oviduct, correspond in orientation with those of the female in accordance with the law of Hallez (30), so that the cephalic end of the egg, being last to leave the ovipositor, is always less deeply embedded and is ordinarily covered with only the rel-

atively transparent cuticle of the plant when ready to hatch.

The effect of light on hatching was studied under controlled conditions by turning the lights off and on at definite intervals and covering the windows in top of the cabinets with a blanket during periods of darkness. The results are illustrated in Figure 11 by plotting the number hatching in two lots of eggs, held at 80° F., that were alternately exposed to darkness and an illumination of about 80 foot-candles. In both lots most of the eggs hatched while the lights were on regardless of whether this period occurred during the daytime or at night.

Effects of light factors were not critically or extensively investigated, but the indications were that the rate of nymphal development is not influenced appreciably by differences in illumination or by ultraviolet radiation. In these tests temperature and humidity were held constant, and higher intensities of illumination could not be used because of the heat factor. A sun lamp suspended within the cabinets directly over the insect cages was used as the source of ultraviolet radiation. The cages were similar to those used in other experiments (Fig. 1) except that the tops were closed with cellophane. At the distance used, 30 inches, the lamp was rated at about one-third that of midday midsummer sunlight as a source of ultraviolet in the portion of the spectrum that is ordinarily biologically effective.

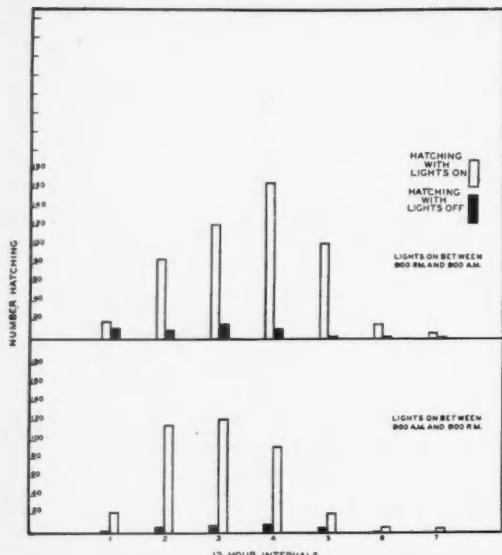


FIG. 11. Number of beet leafhopper eggs hatching in two lots during alternate intervals of illumination and darkness.

At 92° F. and 50% relative humidity the duration of the nymphal stage under continuous exposure to ultraviolet radiation was 13.37 ± 0.10 days for 251 nymphs and under similar exposure to 80 foot-candles of illumination with no ultraviolet, 13.32 ± 0.07 days for 592 nymphs. In another test at 90° and 50% relative humidity, the nymphal stage was 15.16 ± 0.15 days in total darkness and 15.22 ± 0.16 days under continuous lighting of about 40 foot-candles. The difference in time was not significant in either case.

Effect of host plants

The beet leafhopper has been collected from a large number of plant species. Many of these serve only as temporary food plants, but, nevertheless, an imposing list of breeding host plants has been recorded. The more important breeding plants include filaree, *Erodium* sp. (Geraniaceae); many species of saltbush, *Atriplex* spp.; Russian-thistle (*Salsola pestifer* A. Nels.); *Bassia* sp. (Chenopodiaceae); blistereresses, *Erysimum* spp.; peppergrasses, *Lepidium* spp.; and tansymustards, *Descurainia* spp. (Cruciferae).

Haagele (29) listed tumbling orach (*Atriplex rosea* L.), Russian-thistle, *Bassia hirsuta* (L.) Aschers, tumblemustard (*Sisymbrium altissimum* L.), and green tansymustard (*Descurainia pinnata* (Fourn.) Howell subsp. *filipes* (Gray) Detling) as the principal host plants in Idaho. It was later emphasized by Carter (14) that the leafhopper requires a sequence of these plants in which Russian-thistle and tumbling orach are the principal summer and fall breeding plants, and the mustards *S. parviflora* (Lam.) Webb., *D. pinnata* subsp. *filipes* and *Sisymbrium altissimum* are the most important breeding hosts in the spring and

carrying-over hosts for the overwintering adults in the fall and winter. Similar conditions in northern Utah were reported by Knowlton (49) except that *Erysimum repandum* L., *Lepidium perfoliatum* L., and *Erodium cicutarium* (L.) L'Her. were listed as additional host species of importance in this region.

Studies of the nutrition of insects have shown that elementary food substances such as nitrogen or vitamins may influence the rate of growth. Development of the boll weevil (*Anthonomus grandis* Boh.) was observed to be more rapid in young bolls than in old ones (Fenton & Dunnam 23) and to decline as the size of the boll increases (Isely 45). Isely suggested that the observed differences in growth rate may have been due to changes in the nitrogen content of the plant, as it was shown that the percentage of nitrogen declined progressively from squares to large bolls. Peterson & Haussler (70) observed that development of the oriental fruit moth (*Grapholita molesta* (Busck)) was more rapid in peaches than in apples. The larval period of the Indian-meal moth (*Plodia interpunctella* (Hbn.)) was found to be shorter on figs than on prunes or raisins (Hamlin, Reed, and Phillips 32). Hodge's experiments (42) on growth of the differential grasshopper (*Melanoplus differentialis* (Thos.)) have shown that the rate of development of this species was considerably more rapid on some food plants than on others. Seamans & McMillan (81) reared the pale western cutworm (*Agrotis orthodonta* Morr.) on wheat, oats, barley, rye, alfalfa, sugar beets, sweetclover, and several weed hosts. They showed that development was more rapid on the grains and alfalfa than on the weed host plants.

Under experimental conditions considerable difficulty was experienced in maintaining weed host plants in good condition, and frequent transfers of the leafhoppers to fresh plants were necessary. Experimental factors evidently contributed to an excessively high mortality, and for this reason the results may not provide a fair comparison of the viability of nymphs on the different host plants employed.

As shown in Table 11, the plant is evidently not an important factor in the duration of the egg stage, but the time required for development of nymphs varies considerably on different host plants. Many of these data do not show significant differences in the time required for development, but a trend toward slower growth is indicated on less favorable plants, where a high mortality was observed.

MORTALITY

Effects of temperature and relative humidity

Mortality in the egg stage was difficult to determine since the eggs could not be counted at the beginning of the experiments, and at the termination of the tests hatched and unhatched eggs could not be reliably distinguished by the technique used. An unfavorable effect of the temperature extremes of 105° and 65° F. was plainly indicated, however, by the number of nymphs that died with the heads protruding from the plant tissue.

TABLE 11. Duration of the egg and nymphal stages of the beet leafhopper on different host plants at 90° F. and 50% relative humidity.

Host Species	EGG STAGE		NYMPHAL STAGE	
	Insects observed	Time required	Insects observed	Time required
	Number	Days	Number	Days
Sugar beet, <i>Beta vulgaris</i> L.	603	6.27 ± 0.02	423	14.50 ± 0.07
Tumblemustard, <i>Sisymbrium altissimum</i> L.	202	6.35 ± .03
Red stem filaree, <i>Erodium cicutarium</i> (L.) L'Her.			14	15.00 ± .58
Russian-thistle, <i>Salsola Rali</i> var. <i>tenuifolia</i> Tausch	112	6.28 ± .04
Flixweed, <i>Sophia parviflora</i> (Lam.) Webb.	485	6.29 ± .02	35	15.63 ± .20
Blistercress, <i>Erysimum bakeri</i> (Greene) Rybd.	19	16.00 ± .25
Wild lettuce, <i>Lactuca serriola</i> f. <i>integrifolia</i> Bogenh.	16	16.62 ± .53
Peppergrass, <i>Lepidium alyssoides</i> Gray.	15	17.13 ± .52

The nymphal mortality observed in studies of development at different constant temperatures and 50% relative humidity (Table 2) are shown in Figure 12A. The mortality ranged from about 25 to 39 percent at moderate temperatures, but there were marked increases at the more extreme temperatures. Temperatures in a range of about 75° to 93° F. seem most favorable, but large survivals at 95°, 100°, and 105° indicate considerable tolerance of high temperatures, which is consistent with the insect's occurrence in abundance under high temperatures in the desert environment. It was observed that mortality in the nymphal stage at alternating constant temperatures was affected little by sudden temperature changes of rather wide range.

In some years, in Idaho and other northern areas of distribution, the standard shade temperature may fall below 50° F. for considerable periods in March and April during maturation of the spring generation. Such periods of cold, wet weather, which greatly retard development for 1, 2, and 3 weeks or more, appear to be unfavorable for survival of the embryonic and nymphal stages.

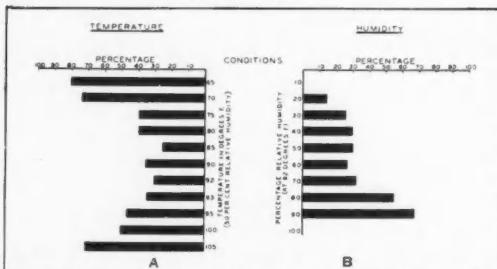


FIG. 12. Mortality of beet leafhopper nymphs: A, Under different temperatures with a constant relative humidity of 50 percent; B, under different percentages of relative humidity, but at a temperature of 92° F.

Other experiments were conducted, in which three periods of exposure to four different conditions of temperature and relative humidity were superimposed on three different stages of development in all combinations, giving $3 \times 4 \times 3$, or 36, different treatments. In these experiments the eggs in early,

medium, and late periods of development, and nymphs of the first, third, and fifth or last instars were exposed for 1, 2, and 3 weeks at temperatures of 40° and 50° F. and relative humidities of 30 and 80% at each temperature. Each experiment on eggs and nymphs consisted of six tests on different dates.

Small potted sugar beet plants were used in studies of the egg stage, since flixweed, the most common spring host plant, did not remain in good condition under the reduced illumination in the cabinets for the period of more than 4 weeks necessary to complete some of the tests. The groups of eggs were obtained by confining 5 ovipositing leafhoppers on different plants for 24 hours at 90° F. Plants containing these eggs were then assigned at random to the different treatments. The nymphs, reared at 90° F., were confined in groups of 25 on flixweed plants and then assigned at random to the different treatments.

The analysis of variance of results for the egg stage shows a significant difference in mortality of the different periods of embryonic development, and a highly significant effect of time of exposure, mortality increasing with later stages of development and with longer periods of low temperatures.

Analysis of variance of data on the nymphs shows highly significant effects of all three factors in which mortality increases with lower temperature, longer exposure, and with earlier stages of development. These effects are illustrated by the following averages of data for the different treatment groups:

°F	% Mortality
50	39.2
40	53.2
Difference required for significance	4.7

Time of exposure, weeks	
1	35.8
2	45.6
3	57.2
Difference required for significance	4.1

Instar	
First	79.4
Third	37.1
Fifth	22.0
Difference required for significance	5.4

Highly significant interactions are also shown between temperature and stage of development, time of exposure and stage of development, and temperature and time of exposure. According to these effects, high nymphal mortality results from relatively shorter exposures with increasingly lower temperatures.

Nymphal mortality increases with lower temperatures and the time of exposure to cold, and both factors have relatively greater effect on the smaller nymphs. With increase in cold, shorter exposures have relatively greater effect on nymphal mortality. Difference in relative humidity at these low temperatures seems to have no appreciable effect on nymphal mortality.

An increased mortality in the egg stage was observed under conditions of high humidity in combination with high temperatures. At moderate temperatures differences in relative humidity had no appreciable effect.

Differences in relative humidity at high temperatures have a marked influence on mortality in the nymphal stage, however. The results included in Table 12 show a progressive increase in mortality as the relative humidity increases from 20 to 90% at 92° F., the correlation coefficient being +0.88. Similar results are shown by data obtained at higher and lower temperatures. Decidedly low relative humidity furnishes a well-defined optimum of atmospheric moisture. Higher percentages of saturation are especially unfavorable at higher temperatures, and at 100° a relative humidity of 90% is near the maximum permitting development through the nymphal stage. It seems probable that these effects result directly from humidity since no evidence of bacterial or fungous infection was observed.

The present results show that relatively low humidities are most favorable, and in this respect the beet leafhopper differs from many other species that have been studied. Relatively high percentages of saturation were found to be more favorable for all stages of the bean weevil (Menusan 62); larvae of the boll weevil (Isely 45); all stages of *Trichogramma minutum* (Riley) and eggs of Angoumois grain moth (*Sitotroga cerealella* (Oliv.)) (Lund 55); and larvae of the onion thrips (MacGill 60). Further data and conclusions, showing low humidities most favorable in the case of different insects or stages, may be found in papers by Peterson (69), Elwyn (22), Davies (18), Zwölfer (95), and Sweetman & Wedemeyer (89).

The data on nymphal mortality in Table 12 have the same correlation with the saturation deficiency as with relative humidity, since these measures are inversely proportional at the same temperature. The results, therefore, cannot be compared to any purpose with differences in saturation deficiency at the same temperature. Nymphal mortality at different temperatures and the same relative humidity decreases from 71 percent at 105° F. to about 30% in the range of 92° to 85°, and then increases with lower temperatures, while the saturation deficiency decreases pro-

TABLE 12. Mortality of beet leafhopper nymphs under different relative humidities.

PROGENY OF OVERWINTERING ADULTS

Temperature (°F.)	Relative humidity	Mortality
92	20	14.29
	30	25.57
	40	29.60
	50	29.94
	60	26.42
	70	32.00
	80	54.67
100	90	63.62
	10	41.33
	90	98.20

PROGENY OF SUMMER ADULTS

90	30	34.60
	50	37.70
	60	58.60
	80	61.10

gressively from the highest temperature to the lowest (Fig. 12). It is evident, therefore, that the combined effects of temperature and humidity on nymphal mortality cannot be expressed in terms of the saturation deficiency.

STUDIES OF THE ADULT

COLORATION

Coloration in the adult stage becomes generally darker as the temperatures at which the insects develop grade downward. At higher temperatures the color ranges from pale yellow at 105° F. to greenish yellow at 100°. Between 95° and 90° the adults are greenish or pale green, and darker areas begin to appear on the wings at 85° to 80°. Color forms having more and darker areas on the wings, vertex, and pronotum are produced at 80° to 75° and at lower temperatures. When reared at high, moderate, or lower temperatures, the insects show a gradation of color characteristic of the "summer, spring, and overwintering forms" in California as described by Severin (82).

Adults of the spring brood in southern Idaho are generally greenish, but in some years they are often marked with darker areas on the wings and are difficult to distinguish from the overwintered insects. During the warmest part of the summer many pale-green and some yellowish individuals are found which resemble those reared at the higher controlled temperatures. Insects that attain the winged stage during colder weather later in the season always bear the dark-colored markings that characterize the overwintering form.

Effects of temperature on coloration of the adult are determined in the last instar probably during

formation of the wings and hypodermis preceding the final molt. This evidently explains the sudden appearance, during cool periods in the spring, and following the first cool weather in the fall, of adults having dark wing markings.

There is considerable random variation in color markings of the nymphs, but those reared at lower temperatures are generally darker especially in the later stages of development. Nymphs hatching from eggs incubated at 60° to 65° F. are grayish white in color, whereas those from eggs kept at moderate or higher temperatures are white.

Coloration appears not to be affected within the adult stage as suggested by Ball (6), since no modifications in color were produced by the exposure of the darker overwintered adults to high temperatures or by subjecting the light-colored summer adults to low temperatures for prolonged periods.

Darker adults could be expected in the field when the maximum temperatures drop below 80° F. for several days in succession, but it is difficult to evaluate exact conditions of varying temperatures necessary to produce a distinct division into dark and light forms, because coloration is influenced by both the time of exposure and the degree of cold in relation to different stages of nymphal development. This is also more complicated by the fact that the color forms intergrade and individual variation may also occur under the same conditions.

Coloration of the adult does not seem to be influenced by any other factors, such as light, atmospheric moisture, sexual development, or age.

The effect of temperature in color aberrations of Lepidoptera is well known, and Knight (48), Genieys (27), Schlotte (79), Parker (66), and Mareovitch & Stanley (59) have shown in other orders that low temperatures produce darker colored adults than higher temperatures.

SIZE

Some seasonal variation occurs in the size and weight of the adult leafhoppers. The lighter-colored adults produced during warmer weather are generally smaller than the darker adults of the overwintering generation that mature in the fall. Larger adults were also produced at lower temperatures under controlled conditions. Males are almost invariably smaller than females that develop at the same temperatures. This difference in weight between the sexes becomes more pronounced with ovarian maturation in the females.

The average weights on the first day of the adult stage for males and females reared through the nymphal stage at different temperatures and 50% relative humidity are shown in Table 13.

Other observations indicating that larger insects are produced at lower than at higher temperatures have been recorded by Standfuss (88), Dewitz (19), Schlotte (79), Titschack (90), Alpatov & Pearl (1), and Menusan (64).

TABLE 13. Average weight, at emergence, of adults of the beet leafhopper reared through the nymphal stage at different constant temperatures and 50% relative humidity.

Temperature (°F.)	Males	Females	AVERAGE WEIGHT	
			Males	Females
100.....	70	70	.794	1.069
90.....	130	130	.841	1.146
85.....	100	80	.865	1.143
80.....	100	100	.891	1.196
75.....	120	130	.897	1.230
70.....	100	100	.903	1.274

SEX RATIO

The sexes are about evenly divided in numbers, with perhaps a slight normal excess of males. The percentage of females was 48.5 in 12,199 adults reared in different experiments. No consistent effect of temperature or humidity on the sex ratio was noted.

Marked differences in the proportion of male and female adults are usually observed in the field. Males require less time for development, and the majority of the first adults to appear in the breeding areas are males. The females apparently have a greater tendency to move when reaching the adult stage, and in such areas the preponderance of males usually continues, whereas in areas populated by dispersal movements the females usually occur in greater numbers. The greater mortality of males increases the sex ratio during the winter, and in southern Idaho almost all the overwintered leafhoppers are females.

FERTILIZATION

Fertilization seems to occur soon after the adult stage is reached and generally before ovarian maturation. Copulation was observed on the first, second, and third days following emergence at 95° F. The mating of older insects was not observed. Females that emerged within a 24-hour period at 90° and that were segregated at the end of this period, laid fertile eggs. Repeated fertilizations are evidently not necessary, since different lots of females used as a source of eggs and nymphs continued to produce fertile eggs throughout life. The observation of Severin (82) that the eggs of virgin females do not hatch was also verified.

THE PREOVIPOSITION PERIOD

Effects of temperature

The length of the preoviposition period ranges from 5 to 6 days in midsummer to 6 or 7 months during the winter. With the occurrence of lower temperatures in the fall there seems to be little, if any, preoviposition development of the darker overwintering form appearing at this time. Development appears to be suspended by the decreased temperatures in the fall and to be resumed following exposure to more extreme cold. This serves to delay oviposition over the winter until conditions permit survival and

development of the next generation. This ovarian quiescence appears to be induced only by seasonal temperature changes in the fall, since both summer and overwintering forms always complete the pre-oviposition period rapidly under controlled conditions at temperatures above 90° F.

Ovarian maturation, like embryonic and nymphal development, is most rapid at about 95° F. (Table 14). Some leafhoppers of both forms complete ovarian maturation at decreased temperatures without exposure to extreme cold, but an increasingly greater proportion of them seem unable to complete development as the temperature is lowered. As was described by Carter (14: 4, 24), the rate of development does not follow the straight-line relationship and ovarian maturation cannot be correlated with ordinary temperature summations.

TABLE 14. Duration of the preoviposition period of the beet leafhopper at different constant temperatures and 50% relative humidity.

SUMMER FEMALES

Temperature (°F.)	Females	Time required	Index rate of development		
				Number	Days
105	25	5.32 ± 0.28	18.80		
100	47	4.66 ± .22	21.46		
95	25	3.96 ± .26	25.25		
94	15	3.87 ± .18	25.84		
92	24	5.00 ± .23	20.00		
90	52	5.31 ± .19	18.83		
85	45	9.93 ± .32	10.07		
80	47	13.83 ± .31	7.23		
75	50	16.74 ± .59	5.97		
70	29	25.07 ± .50	3.99		

OVERWINTERING FEMALES

100	11	2.64 ± .14	37.88
90	32	3.28 ± .08	30.49
85	31	4.13 ± .20	24.21
75	17	5.06 ± .16	19.76
70	12	8.50 ± .28	11.76
65	18	14.56 ± .86	6.87
60	21	21.76 ± .68	4.60

Overwintering females that had been exposed to outdoor temperatures during October, November, and December were able to complete ovarian development at constant temperatures of 60° and 65° F., whereas females of the summer form that had not been exposed to the outdoor temperatures were not able to complete development at these constant temperatures. At higher temperatures the preoviposition period for the overwintering form was shorter than for the summer form, being only a third as long at 75° F. Exposure to cold was evidently a contributing factor, but it is probable that some ovarian development in the overwintering females had taken place before the exposure to constant temperatures.

Durations of the preoviposition period in alternate

12-hour exposures to high and lower temperatures, with some comparisons of relative effects of constant and alternating temperatures on the rate of ovarian development, are shown in Table 13. The time theoretically necessary for development in alternate exposures on the basis of results obtained at constant temperatures was computed as described for data on the egg stage.

TABLE 15. Duration of the preoviposition period of the beet leafhopper (summer form) at alternating constant temperatures and 50% relative humidity.

Temperature (°F.)	Females	Time required	Calculated time		
				Number	Days
110 and 90	11	7.09 ± 0.27	...		
100 and 90	19	4.05 ± .23	4.96	-18.35	
100 and 75	16	6.12 ± .25	7.29	-16.05	
100 and 70	27	6.59 ± .40	7.86	-16.16	
90 and 85	26	7.38 ± .35	6.92	+ 6.65	
90 and 75	16	7.31 ± .23	8.06	- 9.31	
90 and 70	16	9.94 ± .32	8.76	+13.47	

The differences shown in the relative effect of constant and alternating temperatures on the preoviposition period are rather variable, but there is considerable indication that temperature changes produce an acceleration, as occurs in embryonic and nymphal development, because results of most of the tests show a gain in time. It is also shown that leafhoppers of the summer form complete the preoviposition period in a relatively short time in exposures to 90° and 100° F. in combination with lower temperatures. It seems also that ovarian maturation would occur under extremely high maximum temperatures in the field because development was completed in alternate exposures to 110° and 90°. Ovarian maturation was not attained, however, in continuous exposure to 110°.

Effects of humidity

Humidity has an effect on duration of the preoviposition period similar to that described for the nymphal stage. The retarding effect of temperatures above the optimum is slightly accentuated by increase in atmospheric moisture. As is shown in Table 16, at 100° F. the length of the stage apparently in-

TABLE 16. Duration of the preoviposition period of the beet leafhopper under different humidity conditions.

Temperature (°F.)	Relative humidity	Females	Time required		
				%	Number
100	10	37	4.19 ± 0.18		
	50	47	4.66 ± .22		
	90	17	4.71 ± .37		
92	40	18	4.78 ± .26		
	50	24	5.00 ± .23		
	90	20	5.00 ± .36		
		22	5.18 ± .22		

creases with the increase in relative humidity. The data obtained at 92° also show a slight trend of the same sort, but there are no significant differences.

Effects of light

A test of the effect of light on the duration of the preoviposition period was conducted at 90° F. and 50% relative humidity. Insects that developed through the nymphal and preoviposition periods during continuous exposure to the sun lamp were compared with other insects that were reared through the same stages in total darkness. The length of the preoviposition period for the group under the sun lamp was 4.00 ± 0.18 days, and for the group reared in total darkness, 3.73 ± 0.20 days. The difference is not significant.

Fertilization does not seem to have a marked effect on the rate of ovarian maturation. At 90° F. and 50% relative humidity, 5.31 ± 0.26 days was required for preovipositional development of fertile females and 5.57 ± 0.26 days for virgin females. No significant difference is shown.

OVIPPOSITION

Effects of constant temperatures

The leafhoppers used in studies of oviposition were collected in the field, or taken from outdoor cages during the winter, when in the preoviposition stage of development and were held at 90° F. until oviposition began. Excluding those already laying or found to deposit eggs on the first day, a sufficient number beginning egg laying on the same day were divided at random into different groups and placed under different temperatures in controlled cabinets. The total number of eggs laid by each female was ascertained in studies of fecundity. The rates of oviposition under different experimental conditions, however, were not determined until after allowing an adjustment period of 3 days to minimize any effects of previous temperatures, and then the records were taken for only 7 or 10 days in the first part of the oviposition period when egg laying was more uniform among individuals, and the mean rate was more constant over a period of time. The females were always confined singly in cages placed over the petiole and basal portion of the leaves of small potted sugar beet plants, and males were included with them during the preoviposition period at 90°, except in the case of overwintering females, which are normally fertilized in the fall.

The effect of temperature on fecundity of the leafhopper was not investigated throughout the possible gradient, but the oviposition period and the total number of eggs deposited were found to decrease rather sharply at temperatures above 90° F. (Table 17). Egg production seems to be directly limited by higher temperatures, since laying ceases in about 1 day at 115° and in 4 days at 110°, although the females usually live several days longer. Some eggs were also deposited at 120°, but the adults succumbed

TABLE 17. Effects of high temperatures at 50% relative humidity on the length of the oviposition period and the total number of eggs deposited by the beet leafhopper.

Temperature (°F.)	Females	EGGS DEPOSITED		OVIPOSITION PERIOD	
		Number	Average	Number	Average
					Maximum
115	25	4.80	0.93	17	1.08 ± 0.19
110	31	34.61	3.39	70	4.26 ± 0.20
100	50	132.86	11.57	324	15.52 ± 1.14
90	100	229.97	14.92	541	25.71 ± 1.93

in a few hours. The maximum temperature for continued formation and deposition of eggs is near 105°.

When held at 90° F., a group of 67 females deposited 270.45 eggs per female after being removed from outdoor cages in January and February 1932, and an average of 229.97 eggs per female was obtained from a lot of 100 overwintered females in 1934. Maximum depositions of 675, 641, 541, 526, and 508 eggs per female were recorded. Frequency distributions of the data are shown in Figure 13.

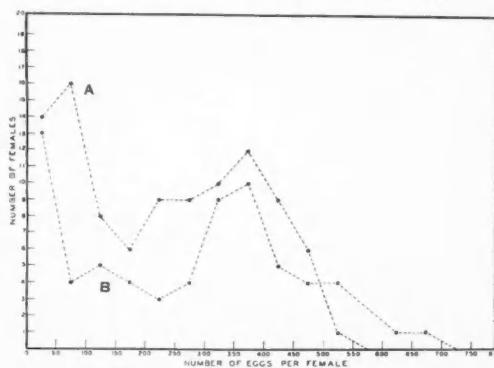


FIG. 13. Frequency distributions illustrating the fecundity of 100 overwintered females of the beet leafhopper: A, In the spring; B, of 67 females removed from outdoor cages in January and February.

The modes of the distributions indicate that the majority of overwintered females may deposit between 300 and 400 eggs under favorable conditions. Probably owing to the occurrence of individuals weakened by exposure to winter conditions, a relatively high mortality early in the tests of females laying fewer than 100 eggs, reduced the average abnormally below the modal number. Stahl (87) observed a maximum deposition of 247 eggs by 1 female and Severin (82) reported an average of 350 eggs from 5 females.

Records of the effects of temperature on the rate of oviposition include data on the egg laying of the light-colored or summer-form adults in August, of the darker overwintering generation late in September (Table 18), and of overwintered adults in March and April (Table 19).

TABLE 18. Oviposition of summer and fall generations of the beet leafhopper at different constant temperatures and 50% relative humidity.

Temperature (°F.)	SUMMER-FORM ADULTS IN AUGUST			OVERWINTERING ADULTS IN SEPTEMBER		
	Females	Eggs	Rate of egg laying ¹	Females	Eggs	Rate of egg laying ¹
	Number	Number	Number	Number	Number	Number
90	16	875	7.81 ± 0.68	18	1,231	9.77 ± 0.56
80	13	456	5.01 ± .51	19	818	6.15 ± .37
70	15	282	2.69 ± .31	17	343	2.88 ± .27
60	15	153	1.46 ± .19	15	147	1.40 ± .10
55	15	72	.69 ± .11	14	83	.85 ± .15

¹ The average daily deposition per female during a 7-day period.

TABLE 19. Oviposition of beet leafhopper of the overwintering generation in the fall and in the spring at different constant temperatures and 50% relative humidity.

Temperature (°F.)	OVERWINTERING ADULTS (September and October)			OVERWINTERING ADULTS (March and April)		
	Females	Eggs	Rate of egg laying ¹	Females	Eggs	Rate of egg laying ¹
	Number	Number	Number	Number	Number	Number
100	21	1,011	6.88 ± 0.47	20	1,712	12.23 ± 0.87
90	18	1,231	9.77 ± .56	45	3,647	11.58 ± .42
85	20	945	6.75 ± .43	14	840	8.57 ± .54
80	19	818	6.15 ± .37	16	696	6.21 ± .57
75	18	526	4.17 ± .27	25	878	5.02 ± .36
70	17	343	2.88 ± .27	25	549	3.14 ± .24
65	19	282	2.12 ± .15	25	391	2.23 ± .18
60	15	147	1.40 ± .10	19	184	1.38 ± .26
55	14	83	.85 ± .15	15	46	.44 ± .16
50	13	54	.59 ± .13	15	9	.09 ± .08

¹ The average daily deposition per female during a 7-day period.

There was a progressive acceleration in the laying rate in all cases as the temperature increased from 55° to 90° F. The oviposition rate of insects of the overwintering generation in the fall was also generally higher than that of the summer-form adults (Table 18), and the rate of egg laying of overwintering insects in the fall was generally exceeded by that of the overwintered females in March and April, after exposure to severe winter temperatures (Table 19). The maximum rate occurred at 90° to 100° F., where the average female laid from 8 to 12 eggs daily. This rate may be maintained for a considerable time, as is shown by an average of about 9 eggs per day for about 26 days by a group of 100 females held at 90° (Table 17). Exceptional females may deposit as many as 30 eggs in a 24-hour period at temperatures of 90° to 100°. With higher temperatures the rate of oviposition is more briefly stimulated and declines more rapidly.

In Figure 14, it is seen that the oviposition rate is not proportional to temperature as would be described by Krogh's formula, but the rising portions of the curves are apparently of the exponential type commonly described by other analytical expressions. In the range of 60° through 90° F. the same data have been fitted to the equations of van't Hoff (B), Arrhenius (C), and Belehrádek (D). Apparently a better fit is obtained with the first two equations. In

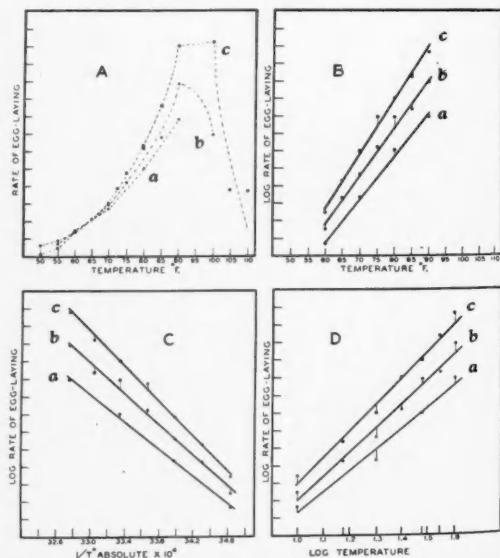


FIG. 14. Rates of egg laying of beet leafhoppers of (a), the summer form in August; (b), the overwintering generation in September; and (c), in March and April when plotted: A, directly against temperature; and according to the equations of B, van't Hoff; C, Arrhenius; and D, Belehrádek.

this case, as was pointed out in a previous discussion of temperature coefficients for insect oviposition (Harries 34), it seems that the percentage increase in rate is closely proportional to the increase in temperature and may be described as well by the more simple van't Hoff formula or preferably by the analogous expression, $y = Ae^{bx}$, which may be used without regard to any theoretical implications associated with the former equation.

Oviposition occurred at temperatures as low as 45° F., but the eggs may have been completely formed in previous exposure to higher temperatures. In this connection Bliss (8) has pointed out in studies of other species of leafhoppers that oviposition may be conditioned directly by temperatures at the time of egg laying and indirectly by the effect of previous temperatures on ovarian maturation of the eggs. The lowest temperature for continued oviposition seems to be near 50°, but this minimum appears subject to some seasonal variation because leafhoppers previously exposed to cold late in the fall and in the winter lay appreciably more eggs at this temperature than those collected in the summer and early fall or after warm weather in the spring. Although some eggs are deposited at more extreme temperatures, continuous oviposition occurs from a slightly lower minimum or through about the same range of temperature as embryonic and nymphal development.

Effects of alternating temperatures

Comparisons of the effects of constant and alternating temperatures were made by exposing different lots of overwintered females continuously to different constant temperatures, or alternately at 12-hour intervals to two constant temperatures. The two sets of tests were run simultaneously. Average rates of egg laying were based on individual records during a 10-day period, after allowing for an adjustment interval of 3 days under the same conditions.

Considerable individual variation occurred in the

TABLE 20. Oviposition of overwintered beet leafhoppers in March and April under alternate 12-hour exposures to different temperatures and 50% relative humidity.

Alternating temperatures (°F.)	Females	Eggs laid in 10 days	DAILY RATE OF EGG LAYING		Deviation
			Observed	Calculated	
105 and 90	20	1,890	9.45 ± 0.32	7.44	+27.02
100 and 90	14	1,464	10.46 ± .67	11.60	- 9.83
95 and 90	20	1,849	9.25 ± .47	9.90	- 6.57
90 and 85	19	1,788	9.41 ± .52	8.10	+16.17
90 and 80	17	1,352	7.95 ± .52	8.52	- 6.69
90 and 75	20	1,887	9.44 ± .36	9.40	+ .43
90 and 70	17	1,595	9.38 ± .63	6.75	+38.96
90 and 65	20	1,823	9.12 ± .39	7.05	+29.36
90 and 60	20	1,535	7.67 ± .43	6.77	+13.29
90 and 55	16	539	3.37 ± .24	4.82	-30.08
90 and 50	17	607	3.57 ± .39	4.65	-23.23
80 and 65	18	742 ¹	5.89 ± .39	5.68	+ 3.70
75 and 65	20	915	4.58 ± .20	4.97	- 7.85

¹In 7 days.

rate of egg laying, and, owing probably to the limited number of insects that could be handled simultaneously the results (Table 20) are rather inconclusive. Since the number of plus and minus deviations are approximately equal, the rate of egg laying is not affected by temperature changes, at least not sufficiently to be demonstrated within the range of experimental error.

Effects of humidity

Studies of oviposition under different conditions of atmospheric moisture were conducted during November and December 1933 with leafhoppers of the overwintering generation that were collected late in the fall. Rates of egg laying were based on individual records of the females for a 7-day period at different humidities, after being held for an adjustment period of three days under the same conditions. All the insects were taken from the same group and were used during the first portion of the oviposition period. The results (Table 21) indicate that the rate of egg laying is not appreciably affected by differences in relative humidity. Since few of the ovipositing females died during the experiments, no significant effect of humidity on mortality of the adults was shown within the 10-day exposure to different conditions,

TABLE 21. Oviposition of the beet leafhopper under different conditions of relative humidity with the temperature held at 90° F.

Relative humidity %	Eggs laid by 50 females in 7 days	Daily rate of egg laying	Number	
			Number	Number
20	5,089	14.54 ± 0.64	5,089	14.54 ± 0.64
30	4,936	14.10 ± .35	4,936	14.10 ± .35
40	4,589	13.11 ± .32	4,589	13.11 ± .32
50	5,152	14.72 ± .42	5,152	14.72 ± .42
60	5,179	14.79 ± .46	5,179	14.79 ± .46
70	4,923	14.07 ± .28	4,923	14.07 ± .28
80	4,737 ¹	13.81 ± .64	4,737 ¹	13.81 ± .64
90	5,254	15.01 ± .52	5,254	15.01 ± .52

¹49 females.

Menusan (63) found that the bean weevil deposited the greatest number of eggs at a relative humidity of 90%. Opposite results were found by Schubert (80) for the beet bug (*Piesma quadratum* (Fieb.)). Other references have been reviewed by Richardson (73) and Uvarov (91).

Effects of light

Oviposition of the leafhopper was not studied extensively in relation to light factors, since preliminary experiments indicated that light did not have any marked effect. A daily rate of 14.83 ± 0.59 eggs was observed under conditions of total darkness. A comparable rate of 14.74 ± 0.65 eggs per day was obtained during continuous exposure to about 40 foot-candles of illumination from ordinary light bulbs, and a rate of 15.57 ± 0.64 under ultraviolet radiation

provided by continuous exposure beneath a sun lamp. The experiments were conducted simultaneously at 90° F. and 50% relative humidity with insects from the same group. The average oviposition rates are based on the number of eggs deposited by 23 to 25 females during a 10-day period. These results show no significant differences attributable to light.

Effects of age

Data obtained in studies of 100 overwintered females at 90° F. and 50% relative humidity (Fig. 15) show that the rate of egg laying gradually declines with age although it is fairly constant during the period of 2 to 3 weeks in which the majority of the eggs are deposited. Oviposition of individual females is continuous at different temperatures and is not subject to cyclical or periodic variations.

Most of the females for which oviposition records were obtained died without showing a definite lapse in egg laying. The average postoviposition period for a group of 100 females was less than 2 days, which indicates that oviposition continues throughout adult life and that egg-depleted individuals would never comprise an appreciable percentage of the population.

Effect of host plants

To obtain some comparison of oviposition on different host plants, laying individuals from the same collection of spring-generation migrants from beet fields in June 1936 were placed on potted sugar beets, beans, and tomatoes, and on several common weed hosts that were transplanted into flower pots from outdoors. The plants were held in the greenhouse for a sufficient time before the tests for any eggs that may have been present to incubate. Tests on different plants were made at the same time at 80° F. and 50% relative humidity. The leafhoppers were transferred to fresh plants each day, and the eggs were counted by clearing the exposed plants in chloral hydrate. The average numbers of eggs per female laid during a 7-day period by 14 to 16 individuals on each species of plants are shown as follows:

	Eggs deposited
Sugar beet	30.1
Tumbling orach	4.2
Tomato	2.6
Russian-thistle	1.7
Flixweed	1.2
Bean	.2
Smotherweed	.2

It is well known that sugar beets are one of the best hosts and that beans and tomatoes are among the poorest, as is indicated here. Unless the adaptation to certain plants must be more gradual, it is difficult to account for the small number of eggs in the weeds, since flixweed and Russian-thistle are favorable hosts and considerable numbers of newly hatched nymphs have been observed on both smother-weed (*Bassia hyssopifolia* (Pall.) Kuntze) and tumbling orach in the field.

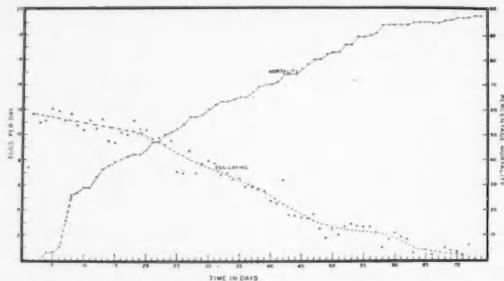


FIG. 15. Average daily oviposition and cumulative mortality of 100 overwintered beet leafhoppers at 90° F. and 50 percent relative humidity.

LONGEVITY AND MORTALITY

Effects of temperature

Leafhoppers collected from wild host plants in the fall were used in studies of the effects of temperature and other factors on the length of adult life. Data on the average survival of some groups of adult females at different temperatures from 30° to 115° F. are given in Table 22. These insects were collected and placed under controlled conditions in November, and small potted sugar beets were used as host plants at all temperatures. Data obtained at below 90° are not strictly comparable with those at higher temperatures, since tests in the two portions of the gradient were not conducted at the same time. It is shown, however, that the length of life decreased to a few days at 110°-115°, was greatly extended with lower

TABLE 22. Average length of life of overwintering females of the beet leafhopper when collected in November and placed under different temperatures.

Temperature (°F.)	Relative humidity	Females	Length of life
			Days
115	50	26	2.24 ± 0.23
110	50	32	9.56 ± .75
100	50	50	16.12 ± 1.15
90	40	100	28.18 ± 2.00
60	50	100	82.86 ± 2.52
50	50	100	73.78 ± 2.84
40	50	100	50.42 ± 2.90
30	—	100	9.66 ± .42

TABLE 23. Comparison of average length of life of male and female beet leafhopper adults at different temperatures.

Temperature (°F.)	Relative humidity	LENGTH OF LIFE, DAYS	
		Males	Females
60	50%	13.78 ± 1.17	82.86 ± 2.52
50	50%	14.01 ± .73	73.78 ± 2.84
40	50%	18.31 ± .70	50.42 ± 2.90
30	—	6.70 ± .20	9.66 ± .42
15	—	3.20 ± .17	14.68 ± .83
10	—	4.49 ± .31	11.94 ± .45

temperatures and decreased metabolism down to 60°-50°, and then decreased markedly again when physical activity and feeding were suspended in exposures at 30°.

Results given in Table 23 show that the females live considerably longer than the males at the same temperatures. Relative values for males and females at the same temperature are strictly comparable, but not those at different temperatures, since the tests were conducted at different times.

Effect of host plants

The length of adult life also seems to be affected by the species of food plants, since females on flixweed survived longer than others from the same collections that were started at the same time on sugar beets. Averages of the length of life on flixweed at 50°, 40°, and 30° F. were 82.55 ± 2.61 , 86.54 ± 4.00 , and 10.41 ± 0.37 days, respectively, in comparison with the corresponding averages on sugar beets at the same temperatures shown in Table 23.

Effects of evaporation and moisture obtained in feeding

During dispersal movements feeding is interrupted when the leafhopper is airborne or distributed where there are no host plants. The insect survives only a very short time without feeding, but the length of life increases considerably with lower temperatures and higher relative humidity, as is shown in Table 24. Similar effects of temperature on the survival of unfed leafhoppers are shown by data in Table 25.

Closely related to the effect of humidity is the

TABLE 24. Longevity of overwintering unfed females of the beet leafhopper under different conditions of temperature and humidity.

Temperature (°F.)	Relative humidity	Saturation deficiency	LENGTH OF LIFE (in days)	
			Average	Maximum
90	%	Mm.		
			1.94 ± 0.08	3.0
			1.38 ± .04	2.5
			1.23 ± .04	1.5
			.94 ± .03	1.5
			.84 ± .03	1.0
			.77 ± .03	1.0
			.59 ± .03	1.0
70	%	Mm.		
			3.02 ± .09	5.0
			2.35 ± .19	5.0
			1.92 ± .10	3.5
			1.49 ± .06	3.0
			1.54 ± .04	2.0
50	%	Mm.		
			16.7	1.28 ± .05
			18.6	1.05 ± .07
			10.56 ± .84	19.0
			7.16 ± .49	11.5
			3.82 ± .34	8.5
50	%	Mm.		
			4.6	4.13 ± .25
			6.4	3.40 ± .23
			8.2	2.96 ± .23
			9.1	2.74 ± .20
				4.5

TABLE 25. Longevity of overwintering females of the beet leafhopper at different temperatures and about 50% relative humidity without host plants and with and without water for feeding.

Temperature (°F.)	LENGTH OF LIFE (in days)			
	With distilled water		Without water	
	Mean	Maximum	Average	Maximum
115	0.64 ± 0.22	1.00	0.06 ± 0.01	.08
110	2.60 ± .16	4.00	.18 ± .01	.21
100	4.04 ± .25	7.00	.35 ± .01	.54
90	4.04 ± .33	6.50	1.03 ± .03	1.50
85	5.28 ± .34	9.00	1.00 ± .02	1.50
80	5.95 ± .70	11.50	1.31 ± .06	2.00
75	7.60 ± .65	12.00	1.50 ± .05	2.00
70	12.28 ± 1.03	24.00	2.30 ± .11	4.00
65	19.00 ± .92	33.00	2.87 ± .15	5.00
60	25.56 ± 1.35	35.00	2.96 ± .16	4.00
50	31.44 ± 3.33	61.00	4.28 ± .20	6.00
40	37.62 ± 1.95	80.00	6.40 ± .65	12.00

matter of the quantity of water available to the insect, either as a part of its food or in the form of contact moisture. As is shown in Table 25, the time of survival without host plants was considerably extended at all temperatures when the insects were supplied with distilled water. The decrease in time of survival of unfed leafhoppers with lower humidities at the same temperature (Table 24) seems to be due largely to the rate of water loss, since Table 26 shows that the survival time was about the same under different humidity conditions when the insects were given distilled water.

TABLE 26. Longevity of overwintering females of the beet leafhopper at 70° F. under different humidity conditions when fed on distilled water.

Relative humidity %	Saturation deficiency (Mm.)	LENGTH OF LIFE (in days)	
		Average	Maximum
90	1.9	4.12 ± 0.22	7
80	3.7	3.80 ± .23	8
70	5.6	3.74 ± .17	8
50	9.3	4.28 ± .28	11
40	11.2	3.66 ± .23	8
30	13.0	4.64 ± .33	11
10	16.7	4.76 ± .39	13

During migration and dispersal movements, and also when the seasonal sequence of favored host plants is unfavorable because of premature drying or delayed germination, almost any species of plant may be used at least as a source of moisture, and it seems probable that such unfavorable food plants as sage brush, *Artemisia tridentata*, Nutt. serve chiefly in this way as temporary or hold-over hosts. Since Table 25 shows that the length of life without host plants may be increased to as much as 80 days at 40° F. when the insects were supplied only with water, late fall or winter precipitation may also be an important factor.

in extending survival when there is delayed or sparse germination of winter host plants.

According to Dalton's law the rate of evaporation from a water surface is proportional to the saturation deficiency of the air. Buxton (11), working with the yellow mealworm, found that water was evaporated from the insect in proportion to the saturation deficiency of the air for a short time and emphasized (12) the importance of this measure of the environment with data on insects by Parker (66) and others. Mellanby (61) found that this was nearly the case with bedbugs.

On the other hand, in studies of 3 species of African locusts, Hamilton (31) obtained different results under almost the same saturation deficiency at different temperatures. Leeson (54) found no direct relation between the survival of unfed fleas and the saturation deficiency at different temperatures, and Maereks (57), with eggs of *Microbracon hebetor* has shown that relative humidity, and not saturation deficiency, determined the temperature limits for a given mortality. Maereks (58) also concluded that the processes involved in loss of water by living insects are too complex to be expressed by a simple physical law. Wigglesworth (94) states that the rate of water loss is governed largely by saturation deficiency, but cites a number of exceptions where insects lose more water in proportion to the saturation deficiency at higher temperatures and less than the expected amount in very dry, or moist, air; he discusses a number of factors, both physical and physiological, that cause such departures from the rule.

Table 24 shows that longevity of unfed leafhoppers increased with higher relative humidity and lower saturation deficiency at each of the three different temperatures. The general increase in length of life at all humidities as the temperature decreased from 90° F. to 70° and to 50° shows that temperature has an independent effect which cannot be expressed in combination with that of atmospheric moisture in terms of the saturation deficiency or evaporating power of the air. According to Wigglesworth (94), this could be due to a more rapid loss of water vapor from the tracheal system with increased respiration, or to greater permeability of the cuticle to water and an increasing rate of diffusion in still air, as the temperature rises. At each temperature (Table 24) the time of survival is disproportionately longer in saturated atmosphere than at lower humidities or greater saturation deficiency. Similar examples are cited by Wigglesworth (94), including instances where certain insects are apparently able to absorb moisture from the air at high humidities. However, the possibility exists in the present case that some moisture may have been condensed and imbibed or that hygroscopic excrement was reingested.

Different humidity conditions for tests shown in Tables 24 and 26 were obtained in sealed desiccators with solutions of sulfuric acid. The leafhoppers were given distilled water by the same methods described

by Fulton and Chamberlin (25), in which small mesh-capped cages were placed on feeding dishes capped with animal mesentery.

Effects of low temperatures

The effects of low temperatures in causing mortality evidently depend on the length of exposure as well as on the degree of cold. Continuous exposures under controlled conditions produced complete mortality of groups of overwintering females in approximately 20 days at 30° F., 15 days at 20°, 11 days at 15°, 6 days at 10°, 2 days at 5°, less than 12 hours at 0°, and less than 5 hours at -4°. The average survival is roughly one-half these specified exposures. These results also seem to agree fairly well with those of Carter (14: 63, 64; Tables 28-33), who found that the overwintering females rarely survived 4-hour exposures to -2° F., -10°, and -14° in a commercial refrigeration plant, or when chilled to 0° or lower in laboratory apparatus.

During the winter the insects are evidently protected to a considerable extent against low or subzero minimum temperatures because the soil-surface temperatures rarely reach levels low enough for sufficient time to approach lethal exposures as determined under experimental conditions. This is shown in Figure 16, where corresponding standard shade and soil-surface daily minimum temperatures during the winter are compared for a 10-year period, 1925-26 to 1934-35, at Twin Falls, Idaho. Although subzero temperatures occurred rather frequently and were as low as -20° F. in January 1930 and February 1933, the temperature of the soil rarely fell below 5° and never reached 0°.

Carter (14: 65, 66, 110) pointed out that the surface of the soil is well protected against extremes of low temperature and that for three winters the soil-surface temperatures did not reach the lethal minimum temperatures determined experimentally. On this basis he was unable to account for differences in survival in hibernation cages and the overwintered or spring populations in 1926-27 and 1927-28 as a direct effect of low temperature, either as extreme cold for a short time or longer durations of more moderate low temperatures. He concluded, however, that "the more frequent the occurrence of temperatures below zero Fahrenheit, the greater the reduction in overwintering populations."

In the action of subfreezing temperatures previously discussed, shorter exposures are lethal or equal periods are more lethal as the temperature decreases, but this seems to depend to a considerable extent on the occurrence of continuous or unbroken exposures, because higher fluctuations of however slight duration appear to counteract the effects of intensity and duration of cold. This effect was observed when two portions of a collection of overwintering females were exposed at the same time in a cabinet at 10° F. The mortality in continuous exposure of one group was estimated by removing and discarding a sample of 50 insects each day, while the

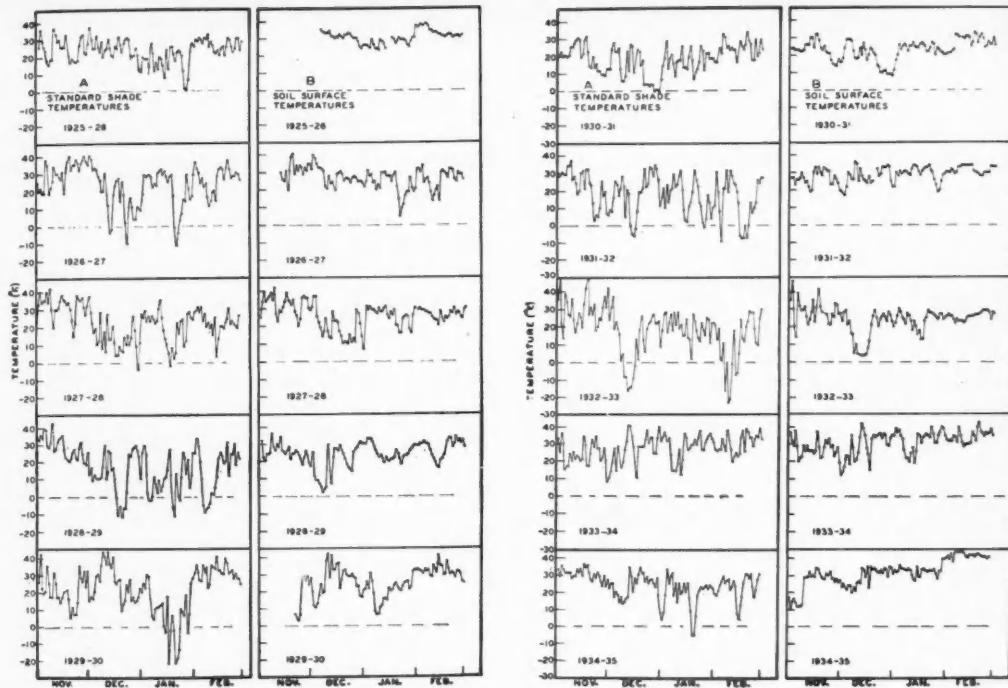


FIG. 16. Comparisons of standard shade and soil-surface daily minimum temperatures for the years 1925-35 at Twin Falls, Idaho.

other group was removed from the chamber for 5 to 10 minutes daily and then replaced after sufficient warming to permit slight life movements by the survivors, so that mortality on successive days could be determined. In continuous exposures the average survival was $4.74 \pm .23$ days, while the second group withstood a much longer exposure of $11.94 \pm .45$ days or a significantly longer total time under the same conditions.

In further studies using both methods of procedure and observation, it was found that effects of the intensity of subfreezing temperatures were counteracted by broken exposures so that the time of survival at lower levels was about the same as at 30° F., where death apparently results from desiccation or only indirectly from cold in preventing physical activity for feeding. The average survival in broken exposures at different temperatures is shown as follows:

$^{\circ}$ F	Days
30	10.16 ± 0.31
25	$10.77 \pm .30$
20	$11.22 \pm .27$
15	$10.11 \pm .28$
10	$10.79 \pm .23$

In exposures broken 5 to 10 minutes daily for examinations, the longevity was also about the same under different relative humidities at 10° F. and the corresponding saturation deficiencies, as is shown by the following results:

% Relative humidity	Saturation deficiency mm.	Days
70	0.5	$12.46 \pm .60$
50	.8	$13.16 \pm .66$
30	1.1	$12.22 \pm .70$
10	1.4	$13.18 \pm .54$

Inasmuch as death apparently comes from loss of moisture by the insects under these conditions, the similar survival with small differences in saturation deficiency at the same temperature seems significant and the results at 10° , 15° , 20° , 25° , and 30° likewise seems reasonable, because there is so little difference in evaporating power of the air at these low temperatures.

Differences in the effect of broken and continuous exposures on the survival at different temperatures are illustrated in Figure 17 by the increase in divergence of the mortality curves at each level as the temperature decreased. Curves showing the effect of continuous exposure are based on different samples of 250 insects taken from the cabinet each day after the different time intervals and then discarded. Curves labeled "broken exposures" are based on data for the same group of 200 females at each temperature that were removed from the cabinet for 5 to 10 minutes on successive days for examination.

The effect of cold periods during the winter therefore appears to depend to an important extent on the daily maximum temperatures, since the intensity of subfreezing temperatures, at least down to 10° F.,

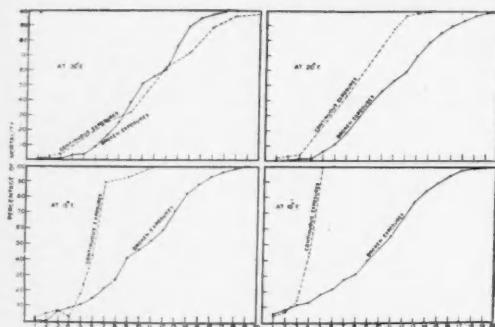


Fig. 17. Relative effects of broken and continuous exposures to cold on mortality of overwintering females of the beet leafhopper in February.

have little effect when relaxed by the diurnal temperature fluctuations. During the winter the leafhoppers are active as temperatures permit, and feeding at intervals is evidently necessary for survival, since the average period endured under controlled temperatures just below the minimum for feeding is only 10 to 11 days and death apparently results from desiccation under these conditions. Extended periods when the ground is covered with snow, or the soil-surface maximum temperatures do not rise above 32° for 1 to 2 weeks or more to permit feeding or imbibition of moisture, would seem to be very unfavorable for survival.

Effects of contact moisture in exposure to cold

In laboratory studies of the effect of contact moisture in exposure to cold the mortality was greater on moist than on dry soil, and the percentages of survival were still lower when the insects were exposed to low temperatures and then warmed to activity under a covering of snow or ice. Overwintering females from outdoor cages when held for 24 hours at 10° F. sustained mortalities of 7.0% when exposed on dry sand, 46.0% on moist sand, and 88.6, 91.2, and 90.4% on moist sand and under 1, 2, and 3 inches of snow, respectively. Similar results, with mortalities of 28, 46, and 93% were obtained in another experiment when overwintering females were exposed at 10° on dry sand, moist sand, and moist sand under about 1 inch of snow, respectively. In these experiments the water drained away and the insects were warmed slowly to activity after the cold exposures. The insects used in the tests were strictly comparable, and the experimental treatment was the same except for the difference in moisture conditions.

Complete mortality occurred when the insects were frozen in ice at 10°.

The effect of winter precipitation on mortality of overwintering leafhoppers has not been adequately investigated in the field, but in comparisons of winter survival in outdoor cages that were kept covered, or exposed by removing the tops during snowfalls, Carter (14: 60, 61, 62) found that mortality of the

insect was increased by contact moisture in wet soil and precipitation.

It seems doubtful, therefore, that high percentages of the overwintering females are very often eliminated at one time by the occurrence of extremely low temperatures or by less severe temperatures for longer periods. Ordinarily winter mortality seems rather to be a gradual wearing down process in which lower temperatures of longer duration, snow cover for extended periods, precipitation, and alternate freezing and thawing chiefly contribute.

Effects of winter conditions observed in sampling studies in 1934-35

Population-sampling studies were continued during the winter of 1934-35 on the Berger plot. The data obtained on the occurrence of overwintering females based on 50 unit samples (except for 49 on March 6-9) taken on two proximate days are illustrated in Figure 18 in relation to the soil-surface minimum temperatures recorded during the winter. The actual estimates with their standard errors are listed as follows:

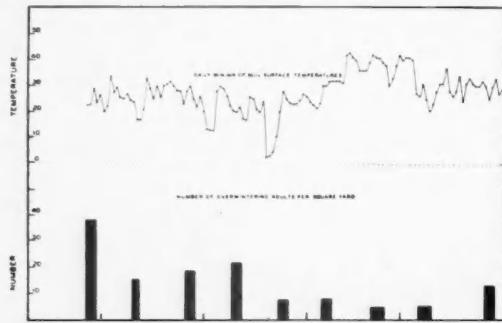


Fig. 18. Number of overwintering females of the beet leafhopper per square yard associated with the daily minimum soil-surface temperatures, Berger, Idaho, 1934-35.

Date of sampling	Female adults per square yard
November 26-28	38.23 ± 6.90
December 10-11	15.53 ± 3.44
December 26-28	18.82 ± 3.76
January 9-11	21.65 ± 5.11
January 23-25	7.53 ± 2.53
February 5-7	8.00 ± 2.16
February 20-23	4.71 ± 1.62
March 6-9	5.27 ± 1.79
March 26-28	12.71 ± 2.52

From November 28 to December 10 the data show a decrease of about 60% in the number of overwintering females, or a statistically significant reduction of 22.70 ± 7.71 per square yard according to the difference in means. Apparently no appreciable mortality occurred during December and the first part of January. Later in January a further significant reduction in number occurred following a cold period, when minimum temperatures near 0° F. were recorded for two nights in succession (Fig. 18). This increase in mortality was evidently produced by low temper-

atures, since experimental work has shown that exposures to 2° - 3° for several hours, obtained by refrigeration, will kill some of the leafhoppers. Late in February and early in March there is another decrease in numbers, which is not statistically significant, however. After some warm weather in March a significant increase occurred that was evidently due to an influx of overwintered females to the observation plot from other points in the vicinity where there were less favorable host plants. Precipitation during the winter was below normal, and the light and temporary snow covers could have afforded little protection from subzero minimum temperatures or caused any appreciable mortality in preventing feeding for sufficient time. Precipitation of 0.58 inch was recorded for December, but this came after the decrease in population shown for December 10 and 11, and apparently had no effect on mortality. In January, February, and March the precipitation was 0.30, 0.29, and 0.03 inch, respectively.

SUMMARY

Studies of the beet leafhopper were conducted at Twin Falls, Idaho, during the years 1932-37. The investigations consist chiefly of laboratory tests of effects of temperature and other factors on development, oviposition, and mortality of the insect with population-sampling studies of its seasonal development and abundance in the field during two seasons.

The average duration of the egg stage ranges from 5.5 days at 100° F. to 43.8 days at 60° . Duration of the nymphal stage increases from 13.0 days at 95° to 75.4 days at 65° . Males complete the developmental stages in slightly less time than is required by the females. Development is very slow at 55° and apparently the minimum effective temperature is between 55° and 50° . Rate of development in the egg and nymphal stages is closely proportional to the temperature between 65° and 95° . In this range of temperature thermal increments of about 200 and 450 day-degrees summed between 58° and 95° are required for completion of the egg and nymphal stages, respectively.

The nymphal stage consists of 5 instars that may be reliably distinguished by the width of the head capsule. The mean headwidth increases about 25 percent between molts, the ratio of increase being fairly constant through successive stages in accordance with Dyar's principle. The duration of the different instars at 90° F. vary from 2.3 to 3.8 days, with the first and the last stages being significantly longer than any of the others at this temperature. The nymphal weight approximately doubles in successive instars and increases from about .04 milligram at hatching to approximately .9 to 1.0 milligram just preceding the final molt. The curve of nymphal growth at a constant temperature is typically sigmoid, but apparently consists of 5 different segments, indicating that the growth ratio differs slightly in the various instars.

In alternate daily exposures to high and low tem-

peratures and under naturally varying temperatures the rate of development is accelerated in comparison with effects of equivalent constant temperatures. The amount of acceleration under variable temperatures apparently differs with the temperatures involved, with the time of exposure at different temperatures, and also with the stages of embryonic and nymphal development completed under different temperature conditions.

Population sampling in 1934 and 1935 shows that 3 generations matured each year. Studies of the annual temperature accumulations over a 10-year period indicate that there are usually 3 generations in southern Idaho, with the last generation sometimes maturing rather late in the fall and possibly limited somewhat in size by insufficient temperatures.

The spring movement to the cultivated areas in May or June begins slowly at first and then increases more rapidly to a peak in from 1 to 3 weeks later, depending on temperatures affecting maturation of the spring generation of leafhoppers in the contributing breeding areas. A high correlation between dates of the beginning of the movement in different years and the recorded temperature increments indicates that temperature is the most important variable governing the time of migration. In years of early migrations advanced by higher spring temperatures early top injury to sugar beets has usually been severe, while beet yields have generally been good when the migration was delayed by lower temperatures.

The rate of embryonic development is apparently unaffected by differences in relative humidity. Nymphal development is slightly retarded with high humidity at unfavorably high temperatures.

Apparently light factors have no important effect on the rate of embryonic and nymphal development. Hatching is stimulated by light after completion of development, and the embryonic period may thus be extended slightly by the occurrence of darkness.

The host plant is not an important factor in the duration of the egg stage, but considerable variation is shown in the time required for nymphal development on different host plants, development being slower on less favorable plants.

Mortality in the egg stage was greater at both temperature extremes for development. Nymphal mortality increases with higher and lower temperatures and with higher humidity. The effects of temperature and humidity on mortality of the immature stages cannot be expressed satisfactorily in terms of saturation deficiency.

In studies of the effects of moderate temperatures just below the minimum for development, differences from 30 to 80% relative humidity at 40° and 50° F. seem to have no appreciable effect on mortality in the egg and nymphal stages. Mortality in the egg stage increases with longer exposures at these temperatures and probably with the decrease in temperature. The late embryonic stages are most susceptible to the effects of cold. Nymphal mortality increases with

lower temperature and with the time of exposure to cold, and both factors have relatively greater effect on the smaller nymphs. With increase in cold, shorter exposures have relatively greater effect on mortality.

The seasonal color forms are determined by temperature conditions. The first adults of the overwintering form appear suddenly after the first cool nights in the fall, and sometimes darker adults of the spring generation that are produced by cooler weather are difficult to distinguish from those of the overwintered generation. As temperatures at which the nymphs are reared grade downward, coloration of the adults ranges from yellowish green at 105° and 100° F. and light green at 95° to darker green at 90° and 85° with darker areas appearing on the wings, vertex, and pronotum at 80° and 75° and lower temperatures. Coloration seems to be largely determined during later stages of nymphal development and probably mostly during formation of the wings and hypodermis preceding the final molt.

Size and weight of the adults is also influenced by temperature. Adults maturing in the fall and spring are generally slightly larger than those appearing during the summer. When reared under experimental conditions the adult weight increases with successively lower temperatures. Female adults are also slightly larger than the males at the same temperatures.

The sexes are about evenly divided under all conditions of temperature and humidity.

Fertilization occurs early in the adult stage and the females continue to lay fertile eggs throughout life although mating was never observed after ovarian development was completed. Fertilization is not necessary for egg laying and apparently has no effect on ovarian development or duration of the preoviposition period.

Average duration of the preoviposition period ranges from about 4 days at 90° F. or 4 to 6 days in summer, to 4 to 6 months during the winter and is apparently governed largely by the maximum temperatures. With the appearance of the darker colored adults of the overwintering generation early in the fall, ovarian development is suspended by the decreasing maximum temperatures, or is at least greatly retarded by smaller increments of higher temperatures. After exposure to more extreme cold has occurred the insect is enabled to complete ovarian development during the remainder of the winter under increments of higher soil-surface maximum temperatures. The rate of preovipositional development seems to be unaffected by differences in relative humidity, except for a very slight retardation with high humidity at high temperatures.

Oviposition begins at about 50° F., the rate is accelerated to about 90°-100°, and then declines at higher temperatures. Effects of the temperature gradient are represented by S-shaped graphs in which data for the rising portion of the curves are well described by van't Hoff formula or analogous expressions where the ratio of increase in rate of egg laying is proportional to the difference in temper-

ature. The oviposition rate is apparently not accelerated by varying temperatures in comparison with results at constant temperatures. The rate of egg laying seems to be unaffected by the quality and intensity of light or by differences in atmospheric moisture. Oviposition continues toward the end of adult life, but the rate of egg laying declines gradually with increasing age. The majority of overwintered females may deposit between 300 and 400 eggs each, and a maximum of 675 eggs was recorded for one individual.

The length of adult life decreases to a few days at 110°-115° F., is greatly extended with lower temperatures and decreased metabolism down to 60°-50°, and then decreases markedly again when physical activity and feeding are suspended in exposures at 30°. Females live considerably longer than males at the same temperatures. The length of adult life is apparently affected by host plants since the insects survived significantly longer on flixweed than on sugar beets. The adult survives but a short time without water either as part of its food or in the form of contact moisture. The time of survival without water increases with lower temperatures and higher humidity and is apparently determined by the rate of water loss from the insect, but the effect of both temperature and humidity factors cannot be expressed satisfactorily in terms of the saturation deficiency. The time of survival is considerably extended when the insects are fed on distilled water, and many unfavorable food plants may serve chiefly in this way as temporary or hold-over hosts in providing moisture.

Effects of subzero temperatures on mortality of the overwintering females depends on the degree of cold and the time of continuous exposure, shorter exposures being lethal or equal exposures more lethal as the temperature decreases. The insects seem highly protected against subzero air temperatures because the soil-surface temperatures rarely reach lethal extremes near 0° F., or low enough levels for sufficient time to approach highly lethal exposures as determined under experimental conditions. Also, the effects of periods of extreme cold would appear to be greatly lessened by temperature rises of however slight duration during the day because under experimental conditions, broken exposures for 5 to 10 minutes daily to permit perceptible life movements counteract the intensity factor of cold so that the time of survival at 25°, 20°, 15°, and 10° is about the same as at 30° where death apparently results only directly from desiccation. Extended cold periods when the ground is covered with snow or the soil surface maximum temperatures do not rise above 32° F. for 1 or 2 weeks or more to permit feeding or imbibition of moisture, would seem to be very unfavorable for survival. Contact moisture as well as thawing and freezing in ice also increases the lethal effects of experimental exposures to cold, and winter precipitation seems generally unfavorable for survival.

Population sampling studies of overwintering leaf-

hoppers on a plot in the desert breeding area show a significant increase in mortality following a cold period in January when the soil surface temperature approached 0° F. for two nights in succession.

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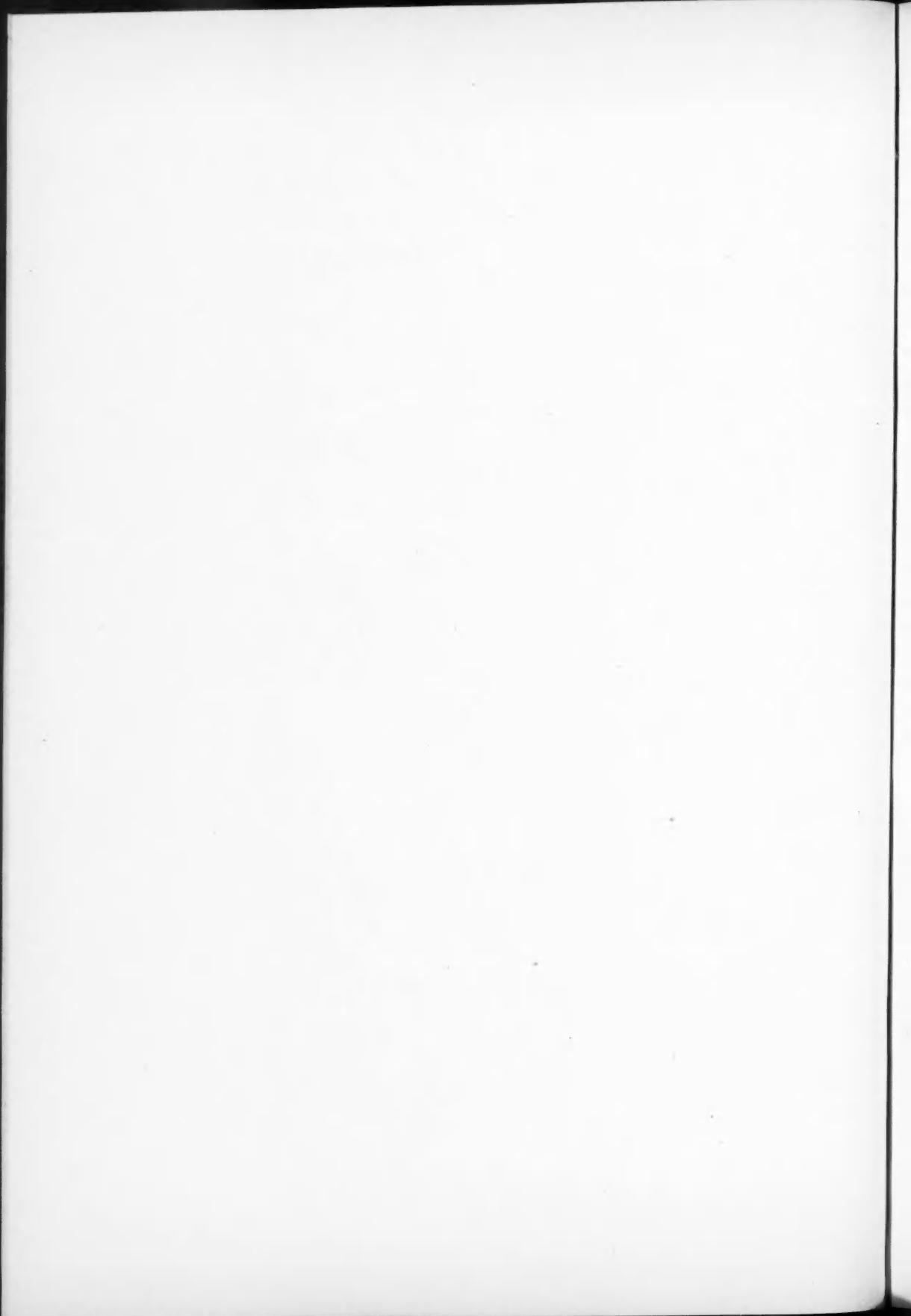
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EFFECT OF CHARCOAL ON CERTAIN PHYSICAL, CHEMICAL, AND BIOLOGICAL PROPERTIES OF FOREST SOILS¹

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EFFECT OF CHARCOAL ON CERTAIN PHYSICAL, CHEMICAL, AND BIOLOGICAL PROPERTIES OF FOREST SOILS

INTRODUCTION

Charcoal is one of the constituents of soils of all forest types in this country, and probably in all forest types in the world, excepting, perhaps, certain tropical rain forests. The amount of charcoal present will vary greatly depending upon the number and intensity of fires common to the forest type. Whenever charcoal is present it may be presumed to exert an influence on the character of the stand beginning with the germination of seed and continuing throughout the life of the organisms. This influence is thought to be due to changes in the physical and chemical properties of the soil and to changes which may occur in the population of competing organisms.

Charcoal is added to forest soils as a result of forest fires, slash burning in forests, controlled burning such as occurs in the southeastern United States, and commercial production of charcoal in hearths located within forests. Gisborne (1932) stated that burned wood in forest soils proves that in the past fire has been of universal occurrence in all present forest types of the United States. According to Plummer (1912) a material resembling charred wood was unearthed in a Pre-Carboniferous sandstone in Michigan. Also, he mentioned that the California big trees (*Sequoia washingtoniana* (Winsl.) Sudw.) indicated by the presence of fire scars the occurrence of great fires as far back as the year 245. The forest area most frequently burned in the United States is the longleaf pine (*Pinus palustris* Mill.) region (Heyward 1939). Within this area a pine stand which had been unburned for 15 years or longer would be a rarity (Heyward 1937). In spite of the amount of burning in this region, less charcoal may be expected to accumulate in the soil than in other regions where fires are less frequent, because the fires of the southeastern forests usually are of the surface-fire class, and chiefly burn organic debris and herbaceous vegetation, whereas in other regions larger amounts of accumulated fuel allow more destructive fires to occur. The presence of charcoal as a result of forest fires may be readily observed in the Appalachian hardwood region.

In Norrland charcoal was present in the upper soil layers in such amounts that Hesselman (1916-17: ei) concluded that it would be difficult to lay out a single square meter in a forest where charcoal could not be found. Similarly, Glinka (1914: 295) found in sections of Asiatic Russia that there was almost no soil profile in which charcoal particles did not occur in the upper horizons.

While the above instances do not give detailed information on the occurrence or amounts of charcoal in the soil, they do indicate the almost universal presence of charcoal in forest soils in the temperate regions.

REVIEW OF LITERATURE

Few studies have been made on the effects of charcoal on soil properties, in spite of its occurrence in forest soils. Many of the references bearing on this problem merely mention some observed effect, no data being presented.

The greatest amount of research on effect of charcoal on soil properties has been done in Italy by various investigators. This work has been concerned mainly with effect of charcoal on growth of agricultural crops.

Verona and Ciriotti (1935) reviewed the work that had been done in Italy since 1927 on vegetable charcoal as a soil amendment. One study concerning the effect of charcoal on the microflora of the soil found that the number of microorganisms was increased, ammonification was increased proportionally to the amount of charcoal added, and that nitrification and nitrogen fixation were slightly increased. Another study indicated that the addition of charcoal to the soil somewhat increased the rate of growth and noticeably improved the quality of beans and corn. The ash content of the charcoal was not entirely responsible for the change as an equal amount of ash, as that contained in the charcoal, did not cause such improvement when added to the soil. Other studies showed improvement of such crops as potatoes, barley, and medicinal herbs when charcoal was added to the soil. One study of the effect of charcoal on the production of tobacco indicated an increase in the size of the crop and greater combustibility of the leaves. It was also found that the improvement of the soil caused by the addition of charcoal lasted at least another year besides the one in which it was added.

Verona and Ciriotti (1935) studied the effect on vegetation of animal charcoal added to soil. Their primary purpose was to compare their results, using animal charcoal, to those of former investigators who had used vegetable charcoal. Their results indicated that:

1. Animal charcoal when added to wheat and bean cultures slightly favored growth but was not so effective as vegetable charcoal.

2. Ammonification of organic substances was favored.
3. Nitrification was favored.
4. Nitrogen fixation was favored.
5. Animal charcoal does not show possession of disinfectant power in such a great degree as vegetable charcoal.
6. Animal charcoal does not show any ability to absorb fluids.

Verona (1938) studied the effect of activated animal and vegetable charcoal on the development of cereal plants and on its disinfecting power. He concluded that addition of charcoal to soil in small amounts favors their growth, as corn plants 29 days old showed a slight weight increase compared to the control. [The heaviest seedlings grown in charcoal weighed 0.723 grams compared with 0.680 grams for the control, or an increase of only 7 percent.]

When liquid from a culture of fungi was added to soil the weights of the seedlings decreased slightly, but this detrimental effect was apparently removed by addition of activated charcoal, which thus shows its germicidal properties.

Perotti and Verona (1938) reported that addition of both animal and vegetable charcoal in small amounts to cultures was beneficial to growth of microorganisms. Charcoal was added to cultures at the rate of 0.01 to 1.0 percent by weight. Some of their tables indicated a slight increase in weight of the organism when from 0.5 to 0.75 percent of charcoal was used, but above 0.75 percent a reduction in weight was observed.

In many of the instances observed, particularly in the two later papers, only small differences can be observed between the check cultures and those treated with charcoal. Because only the average results are reported and no range of data within treatments or standard error given, it is difficult to determine whether or not these differences are statistically significant. Even if they are significant, as the authors believe, these differences may not be of practical importance.

Glinka (1914) claimed that the podsol soils of certain sections of Asiatic Russia have a humus content of 6 and 7 percent resulting partly from the presence of charcoal particles, which cannot be removed, and partly from the virgin condition of the soil.

In Sweden, Hesselman (1916-17: ev) found the flora of charcoal-burning grounds to have a high nitrate content and lists a large number of species found on such areas. He claimed that nitrification must be very active in these charcoal-burning grounds, and an examination showed them to be rich in bacterial life.

Boyce (1925) reported addition of charcoal to soil as a disease prevention measure in a section of the Nag Head nursery in the Forest of Dean, Gloucester. Two-year-old Sitka spruce (*Picea sitchensis* (Bong.) Carr.) seedlings growing in the treated area were killed by heat injury due to excessive surface-soil

temperatures, while no such injury occurred on the untreated areas. This, incidentally, is the first record of heat injury to forest nursery stock in Great Britain.

Salisbury (1925) noted numerous charcoal hearths in the Wyre Forest in England. These hearths vary in age from blackened areas with no vegetation to old areas with a thicket of shrubs and trees. He stated that seedlings developed on the old areas as a result of change in the character of the surface soil. The conditions on an old hearth favoring development of seedlings were believed to be lack of competition, reduced acidity, and increased carbonates and nitrates.

In the United States the most frequently quoted studies concerning the effects of charcoal on soil are those by Retan and others conducted in the forest tree nurseries of Pennsylvania. Retan (1914) mentioned an experiment carried out by Bietzeh in the Mont Alto nursery in Pennsylvania. Production of conifer seedlings had been a failure on the heavy clay soil, and, in an attempt to improve conditions for growth, charcoal residue from old pits was applied to the seed beds. The result was a marked improvement of growth and the two-year-old seedlings on the treated beds were as large as average three-year-olds from untreated beds. A subsequent article by Retan (1915a) emphasized the importance of charcoal in improving water and air content of soil, as well as supplying certain chemical materials to it, and loosening and enriching subsoil.

Data are presented by Retan (1915b) at the Mont Alto nursery, indicating an increase in weight of conifer seedlings in beds treated with charcoal over seedlings from untreated beds. Damping-off of seedlings seemed to be decreased where large amounts of charcoal were added to soil. However, the author stated that more experimentation was necessary before a definite statement could be made concerning the effectiveness of charcoal in controlling damping-off. The exact amount of charcoal used in the treated beds was not indicated, but after plowing and disk-harrowing the area, charcoal was applied in a layer three inches deep. Next, paths were shoveled into the beds followed by a shallow spading. These beds were re-ted to drain quickly following heavy rains, and to hold moisture during dry periods. Another article (Retan 1918) reported the use of a mixture of compost and charcoal screenings as a covering for seedbeds to eliminate caking at the Greenwood nursery in Pennsylvania.

A brief summary of effects of charcoal on the heavy soil of the Mont Alto nursery lists the following benefits (Mont Alto State Forest Tree Nursery 1930): "(a) better soil texture, (b) less damping-off, (c) better germination, and (d) less heaving."

Hartley and Pierce (1917) conducted a single test attempting to control damping-off by addition of charcoal to the soil at the Cass Lake nursery in Minnesota. The experiment proved unsuccessful.

Bates (1924: 143) found that extremes of soil-temperature augmented by the presence of charcoal

favored germination of lodgepole pine (*Pinus contorta* Loud.) seed.

Isaacs (1930) reported maximum surface-temperatures and heat injury to Douglas-fir (*Pseudotsuga taxifolia* (LaMarek.) Britt.) on a yellow mineral soil-surface and a surface blackened by charcoal in the Wind River Valley, Washington. On May 20 the temperature of the charcoal-covered surface rose to 143° F. and the mineral soil to 125° F. A loss of 47 percent of the seedlings occurred on the dark area, but no loss was sustained on the light area. At the end of three days losses of 100 percent of the seedlings were reported on the charcoal-covered area, and only 16 percent on the mineral soil. Again, in an article discussing changes in soils of the Douglas-fir region as a result of fire, Isaacs and Hopkins (1937) emphasized the detrimental effect on establishment of reproduction in areas blackened by charcoal from fires.

Haig (1936: 56) presented data on losses of western conifer seedlings grown on mineral, burnt mineral, duff, and charcoal surfaces due to damping-off organisms. Although losses were higher on charcoal than on mineral areas, the differences were not quite significant.

Holsoe (1936), in an article concerning the Indiana charcoal oven, mentioned the use of charcoal for improvement of nursery soils. In a supplementary note, Mr. Boening, forester in the E.C.W. Camp at Henryville, Indiana, stated that the charcoal was produced for the purpose of improving the structure and moisture-retaining qualities of the heavy clay soil in the state nursery at the Clark County State Forest.

Johnson (1939) used powdered charcoal successfully in control of brown root rot of tobacco. The nature of the causal agent of the disease is not known, but several chemicals having germicidal properties are also effective in controlling the disease. It is suggested that control by charcoal is due to adsorption of the causal agent.

Swenson (1939) studied base exchange capacity and moisture equivalent relations of fresh and weathered conifer and hardwood charcoal in coarse and fine sandy forest soils of Connecticut. He concluded that charcoal increased the base exchange capacity but that fresh charcoal caused a higher base exchange capacity of the soil than did weathered charcoal. No differences were apparent in base exchange capacity of either conifer or hardwood charcoal. The moisture equivalent value of both coarse and fine sand were increased by addition of charcoal, the increase being greater in coarse sand than in fine due to greater modification of physical structure of the coarse sand.

Toumey and Korstian (1942: 281) mentioned charcoal as improving the consistency of a heavy soil and causing better plant growth, and Wilde (1942: 316) stated that charcoal has been advocated for control of damping-off, but definite information is lacking.

Alderfer and Merkle (1944) compared effects of surface application versus incorporation of various mulching materials, including charcoal, peat, manure,

cornfodder, and leaves on certain soil properties. Charcoal seemed to be a less effective mulching material for improvement or maintenance of soil structure than the other materials. During dry seasons surface application of charcoal increased the moisture content, but incorporated charcoal apparently had no such effect. Where charcoal was incorporated in the soil, runoff was less than in the check plots when they were in a dry condition; however, when all plots were moistened to field capacity charcoal was ineffective in reducing runoff.

Summary of information contained in "Review of Literature":

Effect of Charcoal on Physical Properties

1. Establishment of reproduction on areas blackened by forest fires suffers a detrimental effect because of heat injury to seedlings from the high surface-soil temperatures.

2. Water and air content of soil are improved.

3. Mulching or improvement of soil structure is less effectively done by charcoal than by the usual materials such as peat.

Effect of Charcoal on Chemical Properties

1. Acidity is reduced.
2. Content of carbon is increased.
3. Base exchange properties are increased (weight basis).
4. Substances toxic to plant growth may be adsorbed by the charcoal.

Effect of Charcoal on Biological Properties

1. Production of beans and corn is improved.
2. Production of conifer seedlings on a heavy clay is improved.
3. Nitrification, nitrogen-fixation, and ammonification are increased slightly.
4. Microorganisms are increased.
5. Effects on damping-off of forest tree seedlings are uncertain.

MATERIALS AND METHODS

SOILS AND CHARCOALS USED

For the tests of effect of charcoal on soil properties, three forest soils of different textural grades were selected. Such a selection was decided upon particularly because of the probable difference in change of physical properties that charcoal would exert on a coarse-textured soil such as sand, and a fine-textured soil such as clay. A medium-textured soil also was included in the tests. The soils, all belonging to the brown podsolic group, were collected in forests near New Haven, and included a Berlin clay loam, a Maltby sandy loam, and a Merrimac sand.

When the soils were collected, the unincorporated humus and herbaceous vegetation were removed from the mineral soil. The upper four inches of exposed soil were then collected, and all rocks and large roots

removed by passing the sample through a coarse sieve.

The content of organic matter, carbon, nitrogen, and the carbon-to-nitrogen ratio of the fraction less than 2 mm. for each of the three soils are presented in Table 1. Carbon was determined by the Schollenberger method as modified by Allison (1935). Nitrogen was determined by the Kjeldahl method, and organic matter determined by converting content of carbon to organic matter by multiplying by the factor 1.724 (Waksman 1936: 225).

TABLE 1. Percentage of total nitrogen, carbon, the carbon-to-nitrogen ratio, and organic matter of the three soils.

Test	SOIL		
	Berlin clay loam	Maltby sandy loam	Merrimac sand
Total nitrogen.....	0.22	0.07	0.07
Carbon.....	3.5	1.6	1.2
Carbon/nitrogen ratio.....	15.91	22.86	17.14
Organic matter.....	6.1	2.8	2.1

Two kinds of charcoal were used in the tests: conifer charcoal made from pine wood, and hardwood charcoal made from a mixture of Connecticut hardwoods in which oak predominated.

By a series of sievings, two size classes were obtained: 2 to 5 mm., and less than 1 mm.

METHOD OF OBTAINING SOIL-CHARCOAL MIXTURES AND CONVERTING DATA FROM AN OVEN-DRY WEIGHT TO A VOLUME BASIS

Usually moisture content and available nutrients of the soil are expressed as a percentage of oven-dry weight. Kramer (1944: 551), in his article concerning moisture of the soil in relation to plant growth, mentioned that the relationship could be expressed more advantageously on a volume basis. Roots obtain their nutrients and moisture from a certain volume and the size of the volume concerned is about the same whether the soil has a high or low specific gravity. If two soils with a substantial difference in specific gravity hold equal volumes of water for equal volumes of soil, the one with the lower specific gravity would appear to have a greater amount of water present when the results are presented on a weight basis.

The main objection to expressing data on a volume basis is the difficulty encountered in obtaining comparable measurements. For many determinations involving water relationships, such as moisture equivalent and hygroscopic coefficient, and tests of many available nutrients, the samples are collected in the field, brought into the laboratory, and allowed to become air-dry before using. If the data obtained from the samples are to be expressed on a volume basis, the investigator must determine the volume-weight¹

¹ Volume-weight is the weight of dry soil divided by the weight of an equal volume of water.

of the soil in situ or use samples packed from loose soil in the laboratory. It is highly unlikely that the volume-weight of soils packed in the laboratory will be the same as it would be in the field condition. Merely tapping the container with the soil in it will usually give a lower volume-weight than that of the soil in field condition, whereas a severe compacting by mechanical force may give too high a result.

In this paper the data will be presented on a volume basis unless otherwise indicated. A volume basis seems to give better comparative results than a weight basis, as the constituents, soil and charcoal, have such different volume-weights.

In order to determine the effects of charcoal on soil properties, mixtures of charcoal and soil containing 15, 30, and 45 percent charcoal by volume were prepared. Preliminary experiments on moisture relations of soils and charcoals indicated that such a series of mixtures would give linear trends and be suitable for statistical analysis. Mixing was governed by a weight control in order to eliminate differences in packing.

The materials, soil or charcoal, were poured into a 100 cc. graduate, packed with moderate force, and then weighed to the nearest hundredth of a gram. Twenty such measurements were recorded for each soil and each type and size class of charcoal. A table was then constructed of weights of equal volumes of the materials from which the desired volume of any of the materials could be computed. However, when the soil and charcoal were mixed a volume less than the expected 100 cc. was obtained because of the different sizes of the soil and charcoal particles. Therefore, a correction was made so that the actual weights of the constituents used actually brought the mixtures to 100 cc. These values represented the air-dry weights of the 100 cc. mixtures. Next, oven-dry weights of the mixtures were determined so that the oven-dry volume-weights could be computed.

By the application of these figures of oven-dry volume-weights for each mixture, data on soil properties could be converted to a volume basis after the values on an air-dry basis had been determined.

STATISTICAL METHODS EMPLOYED IN ANALYZING DATA

Significance between components of the data was tested by analysis of variance. The terms "significant" and "highly significant" occur throughout this paper. A significant difference means that less than one time in 20 is the difference due to chance, and a highly significant difference means that less than one time in 100 is the difference due to chance. Three types of analysis of variance were used in the tests.

The first test applied to the data was a general analysis of variance, designed primarily to test the significance between treatments. The treatments include such factors as size, type, and percentage of charcoal, and type of soil. The data of physical and chemical properties of soil in most cases have 39 combinations, that is, two types and two sizes of

charcoal, mixed with three soils in percentages of 0, 15, 30, and 45 by volume. Each of the 39 combinations represents one treatment. The data of biological properties have 26 treatments as only two soils are used. If the difference between treatments proves not significant, the conclusion is reached that charcoal has no effect on the soil for that particular property being tested; however, if the difference between treatments proves to be significant, the charcoal affects the properties of the soil, and a second analysis of variance is used to test the single degrees of freedom of the treatments. This is the type of analysis used with the factorial experiment. A discussion of this method is presented by Yates (1937).

Where the charcoal has essentially the same effect on all three soils the 39 treatments were tested together. However, if the charcoal had decidedly different effects on the three soils, the 13 treatments of each soil were treated independently so that three analyses were made, each with 13 treatments, instead of one analysis of the 39 treatments. Linear and quadratic relationships were tested by this method, but only those treatments containing charcoal were included. The controls, soils alone, were not included in the test. When a linear relationship was established for the three different percentage groups of charcoal, a third analysis of variance to test selected linear effects was used. This test determines whether or not a linear relationship exists for the control and three treatments containing the different percentages of charcoal.

The main effects and important interactions between main effects of the various treatments which are significant will be discussed.

The data obtained on the change in reaction of soil with the addition of charcoal were expressed in terms of pH, and these values were also used in the analysis. Soil scientists have not been consistent in their analyses of data of soil reaction. Some have analyzed the values of hydrogen ion concentration; others have analyzed the pH values.

Before an analysis of the data of reaction was undertaken, the proper technique to use was investigated. The conclusion was that pH data are more adaptable to analysis than values of hydrogen ion concentration because the standard deviation of pH data is approximately the same throughout the range of soil reaction tested, a necessary condition for the analysis. If the data of hydrogen ion concentration are analyzed, the standard deviation will vary throughout the range of soil reaction tests, becoming larger when the soil is more acid. Therefore, it is concluded that pH values should be used instead of values of hydrogen ion concentration to test significance between treatments of soil reaction as conducted in this investigation.

Tables of statistical analyses for all tests of significance may be seen in the dissertation entitled "Effect of Charcoal on Certain Physical, Chemical, and Biological Properties of Forest Soils" which is in

the library of Yale University, New Haven, Connecticut.

MOISTURE ADSORPTION BY CHARCOAL

The process of taking up and holding a vapor or liquid by charcoal has been termed "sorption," "adsorption," "absorption," and "chemisorption" by various investigators. The nature of the process involved does not seem to be well understood, which no doubt contributes to the confusion in terminology. Marshall (1935: 38) stated that charcoal is the best known example of a substance having great powers of adsorption for molecules in solution and for gases. Throughout this paper the term "adsorption" will be used when referring to retention of water vapor or water by charcoal, although in citing work by other investigators their choices of terms will be respected.

FACTORS AFFECTING MOISTURE ADSORPTION BY CHARCOAL

The ability of charcoal to adsorb gases and vapors was discovered independently by Scheele and by Fontana in 1777 (Knudson 1924). Since then many data have been collected on this phenomenon and conflicting results and theories have been presented. The experiments have been with various kinds of charcoal, differing in composition in accordance with the nature of the parent material (Wohryzek 1937: 11), method of charring, and manner of activation. Most of the research has been with activated charcoal, as it has great adsorptive powers and has many commercial uses. Knudson (1924) mentioned during the process of charring the mineral content is largely transformed to metallic oxides which also possess individual powers of adsorption. In many instances the ash content of the charcoal was reduced before starting the experiment. Different methods of de-ashing result in charcoals varying in ash content, and this constitutes another factor contributing to the differences in results obtained by the investigators.

The diversified results obtained in the study of water adsorption by charcoal are expressed by King and Lawson (1934) who stated that although the sorption of water by charcoal has been studied more than any other substance it has yielded the most contradictory results. The water vapor is sorbed in infinitesimal amounts at low pressures, and apparently competes with difficulty with impurities on the surface of the charcoal, and small quantities of the sorbed water are held with great force, some of it being held irreversibly.

Large amounts of some gases are adsorbed by charcoal. De Saussure found that one volume of beech charcoal adsorbed 90 volumes of ammonia gas at 11° to 13° C. at 724 mm. of mercury (Mayer 1914: 162). Several other gases were studied by him under the same conditions and the lowest adsorption rate was hydrogen with 1.57 volumes. Water vapor was not studied but it is known that the amount adsorbed would be low compared with many gases.

In studying adsorption isotherms of vapors of

methyl alcohol, ethyl alcohol, water, ammonia, and methyl amine, Knudson (1924) concluded that a different type of phenomenon than adsorption was involved for water than for the other vapors. He suggested that the holding of water vapor by charcoal was probably a result of capillary action rather than of the surface forces of adsorption.

Mayer (1914: 162) also concluded that the ability of charcoal in the soil to hold water was a result of capillary action.

Some conditions which have been studied in relation to adsorption of water and water vapor by charcoal are temperature of carbonization, activation, and age of charcoal.

1. Temperature of carbonization.

Comparisons of ability of charcoal prepared at different temperatures of carbonization to adsorb moisture were made by Violette (Armstrong 1929: 176). The temperatures of carbonization used were from 302° F. to 2,732° F. and a definite decrease in moisture adsorbed with the increase in temperature of carbonization was obtained. At a temperature of 302° F., 100 parts of charcoal adsorbed 20.862 parts of water, and at 2,732° F., 2,204 parts.

2. Activation of charcoal.

The ability of activated charcoal to hold greater amounts of gases and liquids than unactivated charcoal has been known for many years, but the mechanism involved is still not completely understood. It was believed that activation consisted in removing or preventing the formation of complex hydrocarbons which render inactive the points on the charcoal surface. When air or steam is the activating agent these substances are oxidized; chemical activation prevents the formation of the hydrocarbons (Burrage 1933a). A theory was presented by Burrage (1933a) that activation of cellular substances produced pits on the cell walls by the destruction of cellulose, while the lignin, being more resistant, was less altered. These holes or spaces in the surface layer provided more surface area for processes of adsorption.

King (1935) believed that this latter theory did not apply to all substances and concluded that, "The process of activation is probably a complicated one, but consists essentially in the removal by oxidation of poisoning hydrocarbons with a consequent freeing of valencies and formation of active centers, together with a general extension of surface area and increase in size of the original pores owing to the removal of carbon by oxidation."

Hawley (1944: 683) believed ordinary charcoal to consist of a primary charcoal on or in which a secondary charcoal or tar coke had been deposited. The process of activation removes the tar coke leaving the primary charcoal.

3. Age of charcoal.

In 1868 Wallace stated that new charcoal could hold 80 to 100 percent water, but old charcoal could hold only 30 to 45 percent (Burrage 1933b). Thus charcoal loses some of its ability to hold water as it

ages. Burrage explained this difference by assuming the gradual removal of the "spongy mass" on the surface of the charcoal pores where the adsorptive powers reside. Water gradually washes the particles away reducing the adsorptive powers of the charcoal.

If water is held in charcoal merely by capillary attraction rather than by adsorption, as some other investigators believe, a reduction in capillary pore space would appear to be the reason for the difference in ability of new and old charcoal to hold water rather than differences in adsorptive powers.

MOISTURE ADSORPTION BY THE CHARCOAL USED IN THIS STUDY

Adsorption of water vapor by the two sizes of conifer and hardwood charcoal was tested by placing a layer one-eighth inch thick of each in open top jars. The jars were placed in a moist chamber for a period of 14 days. The temperature was about 76° F. and the relative humidity about 90 percent. Seven replications of each size and type of charcoal were tested, and the average percentage of moisture on a weight basis is presented in Table 2.

TABLE 2. Adsorption of moisture by charcoals (dry weight basis).

CHARCOAL		Percentage of moisture	Standard error
Type	Size		
Hardwood	< 1 mm.	9.073	±0.06
Hardwood	2-5 mm.	9.302	±0.26
Conifer	< 1 mm.	10.906	±0.25
Conifer	2-5 mm.	11.452	±0.20

Difference in percentage of moisture adsorbed between the hardwood and conifer charcoal was highly significant, but difference between sizes was not significant for either the hardwood or conifer charcoals. The "t" test was used to determine significance.

The conifer charcoal had greater powers of adsorption of water vapor than the hardwood charcoal when compared on a weight basis. However, this difference was not large. Although no significant differences in percentage of moisture adsorbed existed between the coarse and fine charcoal, the coarse charcoal of both types (hardwood and conifer) adsorbed slightly more moisture than did the fine charcoal.

EFFECT OF CHARCOAL ON PHYSICAL PROPERTIES OF THREE FOREST SOILS

Charcoal was added to three forest soils in order to determine its effect on moisture equivalent, hygroscopic coefficient, and wilting percentage. Ratios of moisture equivalent and hygroscopic coefficient to wilting percentage are also presented. Effect of charcoal on amounts of available moisture in the soil, effect on evaporation, and effect of evaporation on time required to reach permanent wilting were also studied.

Much of the experimentation was conducted in the laboratory and whenever the total number of treatments could not be performed simultaneously, as for example, moisture equivalent determination, a randomized selection of treatments was used.

EFFECT OF CHARCOAL ON MOISTURE EQUIVALENT

The moisture equivalent of a soil was first defined by Briggs and McLane (1907) as that percentage of water which is retained in opposition to a force 3,000 times that of gravity. Briggs and Shantz (1912) introduced the use of a force 1,000 times that of gravity in the determination, and this force is usually used today. This is a useful test, as the moisture equivalent of a soil may be determined easily and quickly in the laboratory and for most soils is approximately equal to the field capacity,² with the possible exception of the coarsest and finest soils (Olmstead and Smith 1938).

The three soils, sand, loam, and clay, and the two types of charcoal in two size classes each were mixed so that the charcoal composed 15, 30, and 45 percent by volume of the total. With the three soils as controls, 39 combinations or treatments were established. Four replications or blocks were run to test the moisture equivalent values of the treatments. The method used was essentially that of Veihmeyer, Israelsen, and Conrad (1924) using 30 grams of the air-dry mixtures, moistening, and centrifuging for 30 minutes at 2,440 r.p.m. One condition was modified, which was to allow the samples to adsorb moisture for 72 hours instead of 24, because from preliminary tests it was found that adsorption of moisture by the charcoal was incomplete for the shorter period and erratic results were obtained.

Percentage of moisture was determined in this and all other instances where moisture relations are involved by weighing immediately after centrifuging, oven-drying at 103° C. to a constant weight, and reweighing. A general analysis of variance brings out highly significant differences between blocks, treatments, and soils within the treatments.

The difference in blocks was due to differences in temperature of the laboratory at the time the tests were run. Two of the blocks were run during early summer and two during mid-summer. The latter two blocks, having moisture equivalent values of 18.22 and 18.15, were significantly lower than the former two blocks, having values of 19.47 and 19.39, because the higher temperature in the laboratory during mid-summer reduced the surface tension of the water; thus more water could be removed by centrifuging at the higher temperature. This variation in moisture equivalent values in relation to temperature has been noted by Briggs and McLane (1910).

The average moisture equivalents of the sand series, loam series, and clay series are 11.69, 16.48, and 28.26, respectively. According to the "t" test, highly

² The moisture content at field capacity has been defined by Veihmeyer and Hendrickson (1931) as, "the amount of water held in the soil after excess gravitational water has drained away and after the rate of downward movement of water has materially decreased."

significant differences existed between each of them.

Significant differences between treatments for the sand series are as follows: Addition of charcoal increases the moisture equivalent value, as the average value for all treatments containing charcoal is 11.83 as compared with 10.11 for the sand alone, the difference being highly significant. Samples containing fine charcoal have a value of 12.26, whereas those containing coarse charcoal have a value of only 11.39. Hence fine charcoal increases the moisture equivalent values of sand more than does coarse charcoal.

The addition of charcoal in amounts equalling 15, 30, and 45 percent by volume of the total sample increases the moisture equivalents at a linear rate of 10.82, 11.77, and 12.89, respectively, the linear relationship being highly significant.

The interaction, type x size, is highly significant, the data being presented in Table 3. The treatments

TABLE 3. Interaction of type x size of moisture equivalent values (sand series).

Type of charcoal	SIZE OF CHARCOAL	
	< 1mm.	2-5mm.
Hardwood.....	12.88	10.67
Conifer.....	11.64	12.12

containing fine hardwood charcoal have a higher value than those containing coarse, but the ones containing coarse conifer charcoal have a higher value than those with fine charcoal. The results obtained for the two sizes of conifer charcoal were unexpected. As equal weights of both size classes of the same type of charcoal adsorb moisture to the same extent, it is to be expected that both types of fine charcoal would have higher moisture equivalent values than the coarse because equal volumes are concerned, and a greater weight of fine charcoal than of coarse makes up the same volumes.

Such a condition was at first believed to be the result of more rapid adsorption by the coarse conifer charcoal than by the others for some unexplained reason, so another series of tests was conducted and the attempt made to eliminate differences in adsorption if such existed. In this test aerosol was used to reduce the surface tension of the water and make adsorption easier for all types of charcoal. All other conditions were maintained the same. Similar results were obtained in this test as in the first one where no aerosol was used to reduce surface tension. The coarse conifer charcoal again retained more moisture than did the fine conifer charcoal, but the fine hardwood charcoal retained more moisture than the coarse hardwood charcoal. The reason for this apparent increase in adsorptive powers or ability to hold water against the pull of gravity by the coarse conifer charcoal is unknown.

The interaction, size x linear relationship, is highly significant. The data are presented in Table 4. The

TABLE 4. Interaction of size \times linear relationship of moisture equivalent values (sand series).

Size of charcoal	PERCENTAGE OF CHARCOAL		
	15	30	45
< 1mm.....	10.94	12.14	13.70
2-5mm.....	10.71	11.40	12.08

addition of fine charcoal to sand increases the moisture equivalent at a greater rate than does the coarse charcoal when added to sand.

Significant differences between treatments for the loam series are as follows: Addition of charcoal increases the moisture equivalent values of the loam. The loam itself has a value of 15.69, but the average value for all treatments containing charcoal is 16.55, a highly significant difference. Fine charcoal increases the moisture equivalent values to a greater extent than does coarse, the values of the treatments being 17.13 for those with fine as compared with 15.97 for those with coarse.

Table 5 presents the interaction, type \times size, which is highly significant. Here again, as was found for

TABLE 5. Interaction of type \times size of moisture equivalent values (loam series).

Type of charcoal	SIZE OF CHARCOAL	
	< 1mm.	2-5mm.
Hardwood.....	17.96	15.19
Conifer.....	16.29	16.75

the sand series, addition of coarse conifer charcoal results in a higher value than that of the fine, and the fine hardwood charcoal has a higher value than the coarse charcoal.

Significant differences between treatments for the clay series are as follows: Addition of charcoal reduces the moisture equivalent. Clay has a value of 31.57, but the average value of all treatments containing charcoal is 27.98, a highly significant difference. Samples containing fine charcoal have a higher value than the coarse: 28.55 as compared with 27.41. Thus coarse charcoal is more effective in reducing the moisture equivalent values of clay than fine charcoal. Addition of charcoal in amounts of 15, 30, and 45 percent by volume reduces the moisture equivalent at a highly significant linear rate from 28.90 to 27.70 to 26.52 with increase in the amount of charcoal.

The only significant interaction, size \times linear relationship, is shown in Table 6. The increase of coarse charcoal in the sample reduces the moisture equivalent at a greater rate than does the increase of fine charcoal.

Linear regressions were indicated as highly significant in two of the analyses by the factorial method for the treatments containing 15, 30, and 45 percent

TABLE 6. Interaction of size \times linear relationship of moisture equivalent values (clay series).

Size of charcoal	PERCENTAGE OF CHARCOAL		
	15	30	45
< 1mm.....	29.78	28.05	27.83
2-5mm.....	29.66	27.35	25.22

charcoal. However, the regression analysis goes a step further and includes the soils without charcoal, a treatment not considered in the former analysis, and the treatments containing charcoal which are grouped by percentage.

The regression lines are plotted in Figure 1. They indicate that addition of charcoal increases the moisture equivalent of sand, slightly increases that of the loam, and causes a substantial reduction in the moisture equivalent of the clay. This linear regression is real and not due to chance, according to the regression analysis.

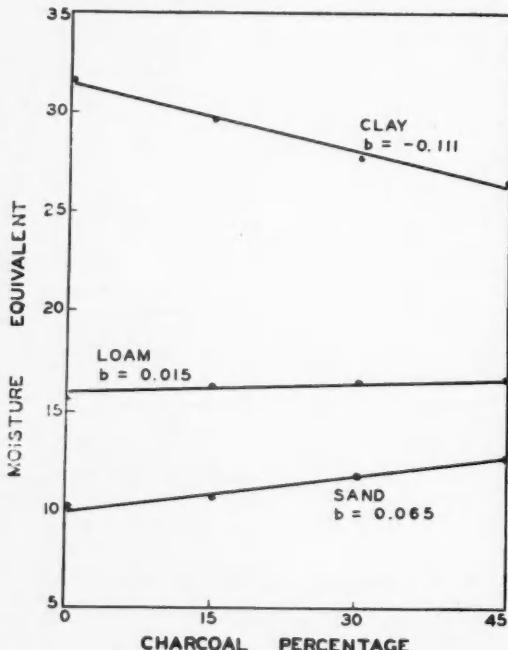


FIG. 1. Effect of charcoal on moisture equivalent values of three soils. Types and size classes of charcoal are grouped by percentage.

EFFECT OF CHARCOAL ON HYGROSCOPIC COEFFICIENT

Hygroscopic water is that water remaining in the soil after capillary water has been removed. This water is held tightly by the soil particles and moves only in the vapor state. The term "hygroscopic coefficient" designates the upper limit of this type of water. It is determined by exposing soil to air

saturated or nearly saturated with water vapor for a suitable period of time.

The method employed was to expose a thin layer of the material to be tested to an atmosphere having a humidity of 98 percent for 5 days. The mixtures were placed in shallow open-top jars and these were set in a glass chamber. A super-saturated solution of $\text{CaSO}_4 \cdot 5\text{H}_2\text{O}$ was placed in the bottom of the chamber which was maintained at a constant temperature of $20.0^\circ \text{C.} \pm 1.0$. By this method a humidity of 98 percent was maintained. After five days exposure the samples were removed, weighed, oven-dried, and reweighed to determine the percentage of moisture.

A block of the 39 treatments composed of three soils and mixtures of the soils, and two kinds and sizes of charcoals added to the soils to comprise 15, 30, and 45 percent of the total volume, were tested simultaneously. The experiment was performed in triplicate.

A general test of analysis of variance indicates significant differences between blocks and highly significant differences between treatments and soils within the treatments. This difference between blocks is very small and of no practical importance. Undoubtedly slight but consistent temperature differences between blocks during the exposure was the responsible factor.

Comparison of the soil series by the "t" test proved each soil series differed from the others in a highly significant manner. The hygroscopic coefficients of the sand series, loam series, and clay series are 1.94, 2.54, and 5.04, respectively.

Significant differences between treatments for the sand series are as follows: Addition of charcoal increases the hygroscopic coefficient, as the average value for all treatments containing charcoal is 1.97 as compared with 1.56 for the sand alone. Sand samples containing hardwood charcoal have a value of 2.03, whereas those containing conifer charcoal have a value of 1.91; thus hardwood charcoal is more effective than conifer charcoal in increasing the hygroscopic coefficient of sand. Samples containing fine charcoal have an average value of 2.08, but those with coarse charcoal a value of only 1.86, the difference being highly significant. The increase of the hygroscopic coefficient was linear with the addition of charcoal, to comprise 15, 30, and 45 percent by volume of the total sample, the values being 1.77, 2.00, and 2.13, respectively.

Significant differences between treatments in the loam series are as follows: Loam samples containing hardwood charcoal have a hygroscopic coefficient of 2.63, whereas those containing conifer charcoal have a smaller value of 2.44. Fine charcoal in the treatments causes high hygroscopic coefficients than coarse charcoal, the values being 2.71 as compared to 2.36. The addition of charcoal in amounts equalling 15, 30, and 45 percent by volume of the total sample increases the hygroscopic coefficients from 2.42 to 2.48 to 2.71, a highly significant linear trend.

The interaction, type \times size, is highly significant, the results being presented in Table 7. Thus the decrease in hygroscopic coefficients from the smaller to the larger size class is greater for the hardwood charcoal than for the conifer when both are added to loam in equal volumes.

TABLE 7. Interaction of type \times size of hygroscopic coefficient values (loam series).

Type of charcoal	SIZE OF CHARCOAL	
	< 1mm.	2-5mm.
Hardwood.....	2.86	2.39
Conifer.....	2.55	2.33

The interaction, size \times linear relationship, is significant and presented in Table 8. The increase in

TABLE 8. Interaction of size \times linear relationship of hygroscopic coefficient values (loam series).

Size of charcoal	PERCENTAGE OF CHARCOAL		
	15	30	45
$\leq 1\text{mm.}$	2.54	2.61	2.97
2-5mm.	2.30	2.35	2.44

hygroscopic coefficient values is greater for the samples containing the smaller size of charcoal than for those containing the larger size, and the slope of a line plotted for the treatments containing fine charcoal would be steeper than that of a line plotted for the treatments containing coarse charcoal.

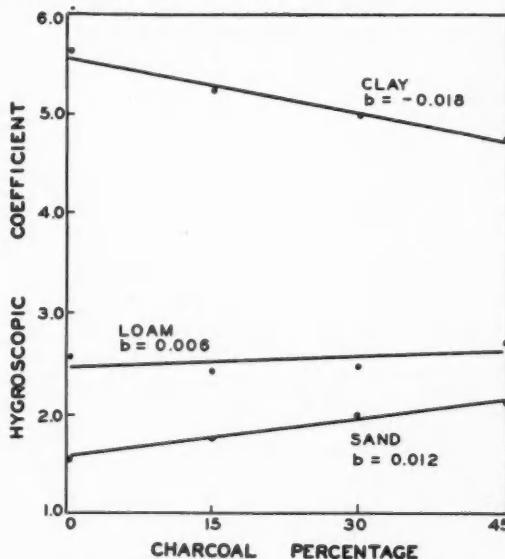


FIG. 2. Effect of charcoal on hygroscopic coefficient values of three soils. Types and size classes of charcoal are grouped by percentage.

Significant differences between treatments in the clay series are as follows: Addition of charcoal to clay decreases the hygroscopic coefficient in a highly significant manner, as the samples containing charcoal have a value of 4.99 and the clay itself has a value of 5.64. Coarse charcoal decreases the hygroscopic coefficient of clay to a greater extent than does fine charcoal. Samples containing fine charcoal have a hygroscopic coefficient of 5.13 and those with coarse charcoal only 4.85. The difference is highly significant. Addition of charcoal in amounts equaling 15, 30, and 45 percent of the total sample causes a linear decrease in the hygroscopic coefficient, the values being 5.23, 5.01, and 4.73, respectively.

Linear regression is highly significant for the three soil series. The regression lines are plotted in Figure 2. They indicate a substantial increase in the hygroscopic coefficient when charcoal is added to sand, a slight increase when added to loam, and a substantial decrease when added to clay.

EFFECT OF CHARCOAL ON WILTING PERCENTAGE

The wilting percentage of a soil is a term used to designate the percentage of moisture in the soil at the time permanent wilting of a plant occurs. The moisture below this amount is unavailable to the plant and that above it is available. The term "wilting coefficient" was used by Briggs and Shantz (1912) for a similar measurement of soil moisture. They defined the wilting coefficient as "the moisture content of a soil (expressed as a percentage of the dry weight) at the time when the leaves of a plant growing in that soil first undergo a permanent reduction in their moisture content as the result of a deficiency in the soil-moisture supply."

Since then other investigators have used terms such as "wilting percentage," "wilting point," and "permanent wilting percentage" to indicate a similar content of moisture in the soil. Hedgecock (1902) used the term "limit of physiological water" to indicate the same condition.

The term "wilting percentage" will be used in this paper. In order to be consistent with other soil-moisture relationships studied, the percentage of moisture in the soil at the time of wilting of the plant will be expressed on a volume basis.

Thirty-nine treatments were tested including three soils and combinations of them with two types of charcoal in two size classes, so that mixtures having charcoal in amounts of 15, 30, and 45 percent by volume were obtained. The experiment was performed in triplicate in the greenhouse. Block I was tested during the month of July 1943, Block II during the month of September 1943, and Block III during the month of January 1944. The soils and charcoals were mixed and placed in cylindrical glass containers having a volume of 175 cc. The soil-charcoal mixtures were then saturated and allowed to stand 48 hours before using. The containers were covered with black paper to prevent light from reaching the roots, and placed in a randomized manner in

wooden flats filled with sand, so that all but one-half inch of the top of the containers was buried. The sand surrounding the containers was watered throughout the test in order to prevent large fluctuations in temperature of the soils and mixtures. Four sunflower (*Helianthus* sp.) seeds were sown in each container, and after germination was complete all the seedlings but one were removed. When the sunflowers had produced three sets of leaves, the surface of the soils was covered with a mixture of paraffin and petrolatum to prevent exchange of moisture between the atmosphere and the soil. The wilting percentage was established by allowing the lower set of leaves of the plants to wilt until they could not be restored to their normal state of turgidity by exposing them in a moist chamber nearly saturated with water vapor. As soon as the percentage of moisture in the soils and mixtures was judged to have attained the wilting percentage, the container was emptied, the upper one-half inch of soil discarded, the roots removed with tweezers, and the soil placed in cans and weighed. This entire operation took only a few seconds and any loss by evaporation from the exposed soils was negligible. Removal of the soil near the surface was necessary as a high percentage of moisture appeared to exist there; this would have caused the wilting percentage values to be too high. Few roots were present in the portion of soil removed.

The general analysis of variance indicates highly significant differences between blocks, treatments, and soils within treatments.

Blocks I, II, and III have wilting percentages of 7.11, 7.38, and 8.01, respectively. The difference between Blocks I and II is significant; the differences between both Blocks II and III, and Blocks I and III are highly significant. As the tests were conducted at different times of the year, it appears that differences in atmospheric conditions are responsible for the differences between blocks.

The temperature of the greenhouse was warmest when Block I was being tested and coolest when Block III was being tested. This difference in mean temperature was approximately 17° F. As the highest wilting percentage occurred during the coldest weather and the lowest during the warmest weather, it is believed that the difference in temperature is responsible for the differences in wilting percentages between blocks.

Highly significant differences exist between each of the three soil series, the wilting percentages of the sand, loam, and clay series being 4.20, 5.74, and 12.57, respectively.

Significant differences between treatments of the sand series follow. Addition of charcoal increases the wilting percentage as the average value for all treatments containing charcoal is 4.27 as compared with 3.33 for the sand alone. Hardwood charcoal is more effective in raising the wilting percentage of the sand than is the conifer, as treatments containing hardwood charcoal have a value of 4.56, but those

containing conifer charcoal have a value of only 3.98. Charcoal added to the sand in amounts equaling 15, 30, and 45 percent by volume of the samples increases the wilting percentage at a linear rate. The values are 3.73, 3.96, and 5.13, respectively.

Significant differences between treatments for the loam series are as follows: Samples containing hardwood charcoal have a value of 6.03 and those with conifer charcoal a value of 5.48; thus hardwood charcoal increases the wilting percentage more than conifer, when equal volumes are added to loam. Addition of charcoal in amounts equaling 15, 30, and 45 percent of the total sample causes a significant linear increase in the wilting percentage, the values being 5.55, 5.68, and 6.04, respectively.

One interaction, type \times linear relationship, is significant, the values being presented in Table 9. Thus

TABLE 9. Interaction of type \times linear relationship of wilting percentage values (loam series).

Type of charcoal	PERCENTAGE OF CHARCOAL		
	15	30	45
Hardwood.....	5.58	5.92	6.59
Conifer.....	5.52	5.44	5.94

the wilting percentage of loam soils containing hardwood charcoal increases at a faster rate than do those containing conifer charcoal when equal volumes of the charcoals are added to the loam at an increasing rate.

Significant differences between treatments for the clay follow. Addition of charcoal to clay decreases the wilting percentage, the difference being highly significant. Samples containing charcoal have an average value of 12.44, whereas those without charcoal have an average value of 14.03. Fine charcoal, when added to clay, decreases the amount of moisture in the soil at the time of wilting more than does coarse charcoal; the values are 12.77 for the coarse and 12.12 for the fine charcoal. Additions of charcoal amounting to 15, 30, and 45 percent of the total volume of the sample reduce the wilting percentage in a linear manner. The values are 12.96, 12.36, and 12.01, respectively. This linear trend is significant.

Linear regression is highly significant for the sand series and significant for the loam and the clay series when both controls and treatments containing charcoal are considered. Regression lines are shown in Figure 3. These three lines indicate mainly that addition of charcoal increases the wilting percentage of sand to a noticeable extent, but that the value for loam is only slightly raised; the value for clay is reduced to about the same extent that the sand is increased.

The data for moisture equivalents and hygroscopic moisture all indicate highly significant differences between treatments containing fine charcoal and those containing coarse charcoal for all three soil series.

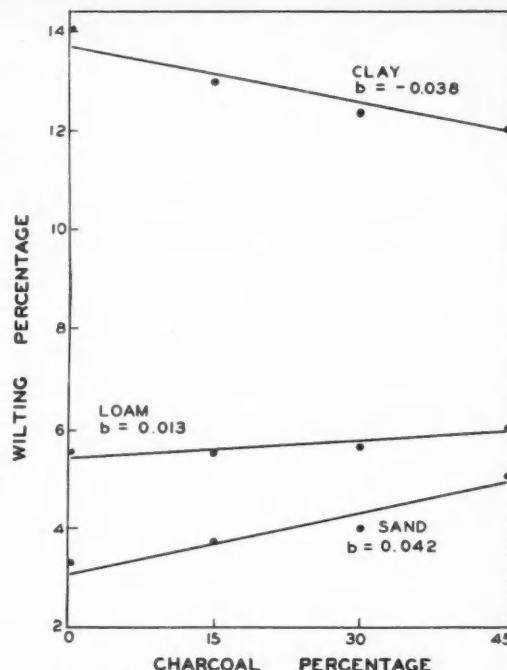


FIG. 3. Effect of charcoal on wilting percentage values of three soils. Types and size classes of charcoal are grouped by percentage.

The treatments with fine charcoal were higher in each instance. This difference is caused largely by the greater amount of fine charcoal than coarse, by weight, in equal volumes. As both sizes of charcoal are able to adsorb approximately equal amounts of water vapor for equal weights, these results seem reasonable.

An examination of the data concerning wilting percentages indicates that the relationship of moisture content between soils containing coarse and those containing fine charcoal is not the same as occurred in the moisture equivalent and hygroscopic moisture data. The difference between wilting percentage of treatments containing the two sizes of charcoal is significant in the clay series, but larger amounts of moisture are held when the coarse charcoal particles are present. Difference between sizes is not significant in the sand series, although here, too, treatments containing coarse charcoal hold a larger amount of moisture at the wilting percentage. In the loam series slightly more moisture is contained in the treatments containing fine charcoal, but the difference is not significant.

Thus, soils containing coarse charcoal tend to hold more moisture when wilting of plants occurs than do soils containing fine charcoal, and this tendency is emphasized when equal weights of charcoal in the soil rather than equal volumes are considered. The reason is that moisture held by the coarse charcoal is

less available to the plants than that held by the fine charcoal. The roots do not penetrate into the charcoal particles, and the moisture in the large granules is apparently held more tightly by capillarity than in the fine. If coarse charcoal did not hold proportionately more moisture than fine at the wilting percentage, values similar to those obtained by the moisture equivalent and hygroscopic coefficient would occur for the wilting percentage values; that is, the treatments containing fine charcoal would be significantly higher than those with coarse charcoal.

EFFECT OF CHARCOAL ON RATIOS OF MOISTURE EQUIVALENT TO WILTING PERCENTAGE

Determinations of ratios of the moisture equivalents to wilting percentage have been determined for many soils of different textures, and although Briggs and Shantz (1912) concluded that the relationship was 1.84,³ most other investigators have obtained no such consistent results. Veihmeyer and Hendrickson (1931) found that ratios of the moisture equivalents to the wilting percentage were quite variable, as high and low ratios were found with sands, loams, and clays.

Wide ranges in ratios of moisture equivalent to amount of moisture in the soil when wilting occurs have been established by Veihmeyer and Hendrickson (1934) and Duncan (1939). The former investigators tested more than 100 soils and obtained ratios from 1.39 to 3.82. Duncan found ratios from 1.57 to 5.65 for three soils from the Duke Forest.

In spite of such differences, the ratio is a valuable one, as moisture equivalents may easily be determined and the approximate wilting percentage established from them. Thus the moisture equivalent divided by 1.84 is an indirect method of obtaining the wilting percentage. Where extremely accurate information is needed the wilting percentage may be established directly, but the method is time-consuming and the exact time when the wilting percentage has been reached is difficult to determine.

The effect of charcoal on the ratio of moisture equivalent to wilting percentage was tested by obtaining ratios for each treatment in each block. The value of each treatment in the first three blocks of the moisture equivalent tests was divided by the value of the corresponding treatment in the block of the same number of the wilting percentage tests. A general analysis of variance indicates that differences between blocks, treatments, and soils within treatments are all highly significant. The difference between blocks of the ratios largely reflects differences between blocks of the wilting percentages. The ratios for the soil series are:

Sand series 2.91
Loam series 2.93
Clay series 2.29

The sand and loam series differ significantly from each other, but the clay series differs in a highly sig-

nificant manner from each of the other two. Hence a lower moisture equivalent in relation to the wilting percentage is indicated for the clay series than for the other two.

Three main facts concerning the effect of charcoal on the ratios are brought out by the general analysis of variance. First, addition of charcoal, when the types, size classes, and percentages are grouped, has no effect on the ratios obtained for the three soils. Second, all the treatments containing conifer charcoal have higher ratios than do those containing hardwood charcoal, although the difference is significant only in the sand and loam series. The ratios for the sand series are 3.10 for the treatments containing conifer charcoal and 2.70 for those containing hardwood charcoal; for the loam series, 3.07 for treatments when conifer charcoal has been added and 2.81 when hardwood charcoal has been added. No significant differences between types of charcoal in the treatment existed in the moisture equivalent tests. In the wilting percentage tests the treatments in all three soil series containing hardwood charcoal had higher values than those containing conifer charcoal, although only two of the series, sand and loam, were significant. Thus, due to lack of differences of moisture equivalents between treatments containing the two types of charcoal, but higher wilting percentages in the treatments containing hardwood charcoal, a larger ratio is obtained for the soils when conifer charcoal has been added.

Third, the treatments containing fine charcoal have higher ratios than those with coarse charcoal for all three soil series. The difference between ratios of 3.13 for the fine and 2.67 for the coarse charcoal in the sand series is highly significant; the difference between 2.40 for the fine and 2.21 for the coarse charcoal in the clay series is significant. This difference between ratios reflects the higher moisture content in the soils containing coarse charcoal when the wilting percentage is reached than occurs between types in the moisture equivalent data.

EFFECT OF CHARCOAL ON RATIOS OF HYGROSCOPIC COEFFICIENT TO WILTING PERCENTAGE

Another indirect method of determining the wilting percentage of the soil is by use of the hygroscopic coefficient. However, this method is rarely used as the value of the coefficient depends largely upon the method used in its determination. Briggs and Shantz (1912) gave the following relationship between the hygroscopic coefficient and the wilting percentage:

$$\text{Hygroscopic coefficient} = \text{wilting coefficient} \\ 0.68 \pm 0.012$$

A similar method of obtaining this ratio was used as in the determination of the ratio discussed in the preceding section, the only difference being the substitution of the hygroscopic coefficient for the moisture equivalent. A general analysis of variance indicates highly significant differences between blocks, treatments, and soils within treatments. The differ-

³ wilting coefficient = $\frac{\text{moisture equivalent}}{1.84 (1.0 \pm 0.007)}$

ence between blocks of this ratio is largely a reflection of differences between blocks of the wilting percentage tests.

The ratio of hygroscopic coefficient to wilting percentage for the soil series follows:

Sand series 0.48

Loam series 0.44

Clay series 0.40

Differences between series by pairs are all real and not due to chance.

Two principal facts are brought out by the analysis of variance. First, addition of charcoal has no effect on the ratio. Second, the ratio for the treatments containing fine charcoal is larger than that for the coarse for all soil series, the difference being highly significant in the sand series and loam series, and significant in the clay series. These ratios are presented in Table 10. These differences in ratios

TABLE 10. Ratio of hygroscopic coefficient to wilting percentage values for soils containing two different sizes of charcoal particles.

Soil	SIZE OF CHARCOAL	
	< 1mm.	2-5mm.
Sand.....	0.52	0.43
Loam.....	.47	.42
Clay.....	.42	.38

between soils containing the two different size classes of charcoal are caused by the greater amount of moisture retained in the coarse charcoal than in the fine when the wilting percentage is reached, as compared with the differences in amount of moisture held by the same two size classes of charcoal at the hygroscopic coefficient.

Charcoal when added to the soil will have no effect on either of the above-mentioned ratios if fine as well as coarse particles are added, but if the charcoal added consists wholly or largely of coarse particles, the ratios will be lowered.

EFFECT OF CHARCOAL ON AVAILABLE MOISTURE IN THE SOIL

The effect of charcoal on amount of available moisture in the three soils was determined by differences between moisture equivalents and the wilting percentages of the soils and soils containing charcoal. No distinction was made between types and sizes of charcoal as the values were obtained from the regression lines of moisture equivalents and wilting percentages (Figures 1 and 3). Actually, the moisture in the soil available to the plant is considered to be that between field capacity and the wilting percentage. Veihmeyer and Hendrickson (1931) presented data showing, "that the moisture equivalent can be used to indicate the field capacities of deep, drained soils with no decided changes in texture or structure, with moisture equivalents ranging from about 30 percent down to about 12 or 14 percent." They found that

below 12 percent the moisture equivalent seems to be lower than field capacity. Edlefsen and Anderson (1943: 33) also stated that for most soils the moisture equivalent is a fairly accurate method of evaluating field capacity.

The soils used in this experiment had moisture equivalents ranging from 7.54 to 33.81 on a weight basis. This range is somewhat greater than that suggested by Veihmeyer and Hendrickson, but as differences in available moisture between soil and soil-charcoal mixtures which are not extremely large are concerned, and not differences in available moisture between soils, satisfactory comparative results should be obtained. The results of available moisture in the soils and soil-charcoal mixtures are presented in Table 11. From this table it is evident that addition

TABLE 11. Effect of charcoal on percentage of available moisture in soils. (Volume basis. Sizes and types of charcoal combined by percentage.)

Soil	PERCENTAGE OF CHARCOAL ADDED			
	0	15	30	45
Sand.....	6.7	7.1	7.5	7.9
Loam.....	10.6	10.6	10.6	10.6
Clay.....	17.8	16.6	15.4	14.2

of charcoal to sand increases available moisture to a limited extent, that it has no effect on loam, and that the amount of available water is reduced in the clay.

EFFECT OF CHARCOAL ON RATE OF EVAPORATION OF MOISTURE FROM SOIL

In order to test the effect of charcoal on the rates of evaporation of moisture, soils and mixtures of soil and charcoal were set up in the greenhouse. The samples were moistened and loss of moisture by evaporation measured.

The three soils, sand, loam, and clay, were mixed with hardwood and conifer charcoal of less than 1 mm. and from 2 to 5 mm. sizes in such a manner that the charcoal occupied 15, 30, and 45 percent of the volume. These combinations with a control included for each of the soils make a total of 39 samples or treatments. The soils and mixtures were placed in No. 2 tin cans which have a volume of about 600 cc. The cans were filled to within three-eighths inch of the top. All treatments were moistened with 225 cc. of tap water, which is about the amount required to saturate the volume of sand used, and then weighed. After adding the water the cans were covered for two days to allow moisture distribution to attain an equilibrium. Next, the cans were reweighed and in some instances slight amounts of water had evaporated and were replaced. The treatments were then placed in a randomized manner on a bench and evaporation allowed to proceed for a 30-day period. Two blocks or duplicate replications of all 39 treatments were tested. The first block was tested from July 1 to July 31, 1944; the second one

from August 4 to September 3, 1944. Essentially the same method was used by Fuestal and Byers (1936) in their work with peat and soil mixtures.

A general analysis of variance indicates highly significant differences between blocks and between treatments. The samples in Block I lost an average of 195.9 grams; those in Block II lost an average of 198.4 grams. The difference is to be expected as the two blocks were not tested simultaneously, and merely indicates that conditions were more favorable for evaporation during the testing of Block II.

The significant differences between treatments are as follows: Highly significant differences exist between the controls and treatments containing charcoal. The controls lost an average of 203.4 grams of water and the treatments containing charcoal lost an average of 196.6 grams. Thus addition of charcoal reduced slightly the loss of moisture by evaporation.

Differences in loss by evaporation between the three soil series are highly significant. The results are as follows:

Sand series 210.1 grams
Loam series 205.4 grams
Clay series 175.8 grams

This illustrates that the finer the texture of the soil the slower the rate of evaporation when equal amounts of water are concerned.

Only one interaction is significant, that one being [(loam + sand) vs. clay] \times linear relationship. The results are as presented in Table 12. The difference

TABLE 12. Interaction of [(loam + sand) vs. clay] \times linear relationship of loss of moisture by evaporation.

Soil	PERCENTAGE OF CHARCOAL		
	15	30	45
Loam + sand	210.7	207.6	203.2
Clay	177.2	175.1	174.0

between percentage classes is greater for the loam and sand than for the clay. Hence addition of charcoal to the sand and loam tends to reduce the evaporation to a greater extent than it does when added to clay.

The fact that the interaction (loam + sand) \times linear relationship is not significant indicates that both the sand and loam are affected to about the same extent as regards loss of moisture by the addition of charcoal.

Smoothed curves showing the amount of water evaporated from the sand and charcoal mixtures are presented in Figure 4, and a similar set of curves showing amount of water evaporated from the clay and charcoal mixtures are presented in Figure 5. Slight differences actually occurred in moisture loss during the first few days, but the differences are so slight that they cannot be differentiated by curves at the scale used. These curves emphasize chiefly two results already noted in the discussion of the data.

First, a decrease in loss of moisture by evaporation with the addition of charcoal to soils; and second, the greater ability of charcoal to decrease loss of moisture by evaporation in light sandy soils than in heavy clay soils.

The curves for the two soils also indicate that

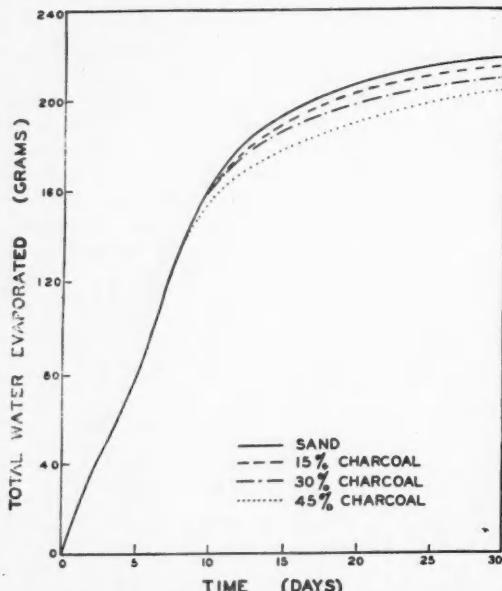


FIG. 4. Effect of charcoal on evaporation of moisture from sand. Types and size classes of charcoal are grouped by percentage.

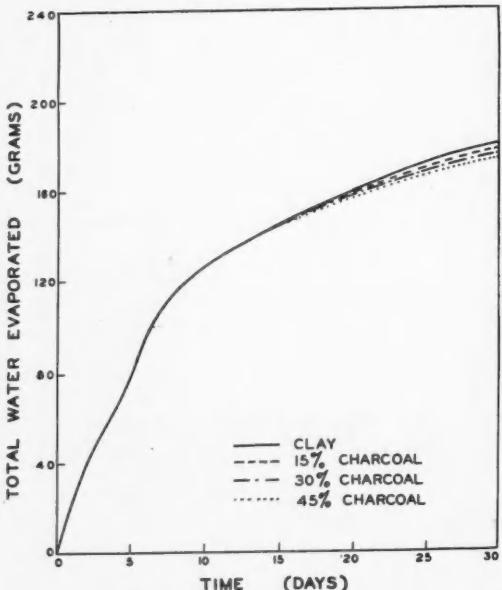


FIG. 5. Effect of charcoal on evaporation of moisture from clay. Types and size classes of charcoal are grouped by percentage.

charcoal is not effective in decreasing loss of moisture by evaporation during the first eight days when added to sand, and during the first 15 days when added to clay.

Although the actual data obtained will apply only when conditions are identical to those existing during the test, it can be concluded that under shaded conditions in nature, charcoal in the soil will reduce the rate of evaporation when the moisture content is low, and that it will reduce it more effectively in a sandy than in a clay soil.

EFFECT OF EVAPORATION ON TIME REQUIRED TO REACH THE WILTING PERCENTAGE IN SOIL AND CHARCOAL MIXTURES

The above data concerning the effect of charcoal on physical properties of forest soils have indicated, among other things, that charcoal increases the wilting percentage of coarse-textured soils, decreases the wilting percentage of fine-textured soils, and reduces the rate of evaporation for the three soils, under greenhouse conditions. If evaporation for all treatments were equal (a false assumption), the wilting percentage of the fine-textured soils containing charcoal would be reached later than that of the soils themselves, where equal amounts of water are concerned, because of the reduced wilting percentage when charcoal is added; but the wilting percentage of coarse-textured soils containing charcoal would be reached more quickly than that of the soils themselves, as charcoal increases the wilting percentage of such soils.

Because charcoal reduces the rate of evaporation the wilting percentage of the clay itself will actually be reached sooner than that of clay containing charcoal; but as it was not known whether the reduction in evaporation caused by charcoal was enough to offset the increased wilting percentage of the sand and loam, and allow available soil moisture for equal periods of time, an investigation was conducted with the data available. All three soils were tested.

The data on loss of moisture by evaporation for all 39 treatments were converted to percentage of moisture in the soil, on a volume basis, for the entire 30-day period during which the evaporation test was conducted. Curves were then drawn for each treatment. The wilting percentage, on a volume basis, was located on the curve, and from this the number of days required to reach the wilting percentage was determined. Figure 6 is a sample to indicate the method used. The curve "A" represents the percentage of moisture on a volume basis contained in the treatment being tested. The wilting percentage, "B," of the same treatment is found on the ordinate which indicates moisture content. A line parallel to the abscissa is drawn to intersect the curve. Next, a line parallel to the ordinate at the point where the curve was intersected is drawn downward to intersect the abscissa. The number of days, "C," is read off the scale at the point of intersection. For the conditions shown, the treatment has a wilting percentage of 4

percent (point "B"). The curve representing the moisture contained in the soil is intersected, and the number of days at this point is 22 (point "C").

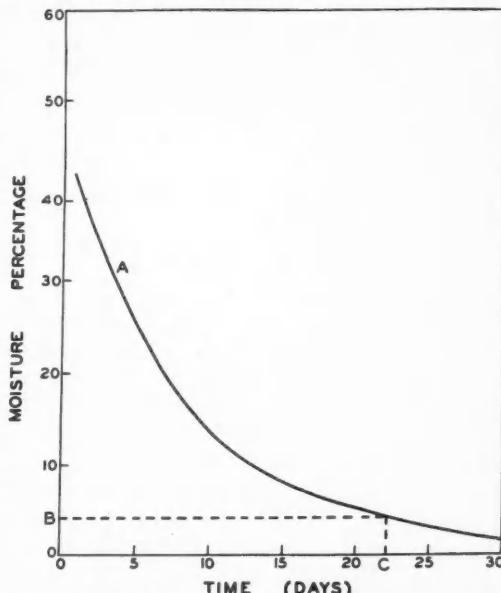


FIG. 6. Sample curve to indicate method of determining number of days required for evaporation to reduce moisture content to the wilting percentage. A. Percentage of moisture in soil (volume basis). B. Wilting percentage of treatment (volume basis). C. Number of days required for evaporation to reduce moisture content to the wilting percentage.

In a like manner data of number of days required to reach the wilting percentage were obtained for the 39 treatments. Highly significant differences exist between treatments without charcoal and those with it; the soils themselves require 17.3 days to reach their wilting percentage, and the soils with charcoal added require 19.2 days. Thus addition of charcoal to the soil increases the number of days before the wilting point is reached under the conditions of evaporation studied in the experiment.

A highly significant linear relationship exists between the percentages. The addition of charcoal in amounts of 15, 30, and 45 percent by volume increases the length of time before the wilting percentage is reached, the average number of days being 18.2, 19.5, and 20.0, respectively.

The use of equal volumes of water in soils and soil-charcoal mixtures for measuring evaporation loss may be justly criticized. Addition of charcoal to a coarse-textured soil increases the field capacity and, under actual conditions, loss from evaporation would start when the moisture content of the soil was somewhere near that point; at least any loss of gravitational water by evaporation would be of no concern. Then, instead of starting the evaporation test of all treatments with equal amounts of water, amounts

corresponding to the field capacity could be used. However, even in coarse-textured soils, charcoal increases the number of days required to reach the wilting point because of the reduction in evaporation caused even when equal amounts of water were used. So, if amounts of water used approximated field capacity, larger amounts of water would have to be used in the treatments containing charcoal in the sand series and loam series, and an even longer time would be required to reach the wilting point of those two soils with incorporated charcoal. For this reason, the length of time required to reach the wilting point is conservative for sand and loam.

With clay, however, the field capacity is reduced by addition of charcoal and much less conservative results would be obtained if amounts of water equivalent to field capacity were used rather than equal amounts.

It must be remembered that the results obtained apply only to conditions approximating those under which the evaporation experiment was conducted. Similar conditions in the field would require the presence of bare mineral soil, no direct sunlight, and no vegetation, a highly unlikely situation.

Actually, loss of water from plants by transpiration, color of surface-soil, intensity of direct insolation, and thickness of unincorporated organic matter would have more effect on loss of water and time required to reach the wilting percentage of the soil than would charcoal incorporated in the soil. With these other factors, especially transpiration, taken into consideration, probably the wilting point of coarse-textured soils containing charcoal would be reached quicker than that of the soils without incorporated charcoal. The coarse-textured soils containing charcoal have higher wilting percentages than the soils themselves. Loss from transpiration would be about the same whether or not charcoal was present, and, where equal amounts of water were concerned, the wilting point would be reached first where charcoal was present. As charcoal reduces the wilting percentage of fine-textured soils the reverse tendency would be expected; a shorter time would be required to reach the wilting point of the fine-textured soil itself, than when charcoal was incorporated in it, as long as loss of equal amounts of water by transpiration is concerned. Hence available moisture would be present for a shorter period for the clay itself than for clay containing charcoal.

However, the tendency of charcoal to reduce evaporation, and to reduce it to such an extent that a longer time is required for the moisture content of the soil to reach the wilting point when other factors are somewhat controlled, is indicated.

EFFECT OF CHARCOAL ON CHEMICAL PROPERTIES OF THREE FOREST SOILS

Charcoal was added to three forest soils in order to determine its effect on acidity, antiacid buffer capacity, base exchange properties, and nutrients

available to forest tree seedlings. Also, the effect of the ash content of charcoal on antiacid buffer capacity was studied, and buffer capacity of the charcoal was compared with that of leaves of some forest trees.

CHANGES IN SOIL ACIDITY BY ADDITION OF CHARCOAL

Three soils, sand, loam, and clay, were mixed with two types of charcoal, hardwood and conifer, in less than 1 mm. and 2 to 5 mm. sizes. The amounts of charcoal mixed with the soils were 15, 30, and 45 percent, by volume. Mixtures of all possible combinations of the charcoals and soils plus the three individual soil checks total 39 treatments.

Air-dry mixtures of the soils and charcoals equivalent to five grams on an oven-dry basis were placed in flasks to each of which was added 100 cc. of distilled water free of carbon dioxide. The flasks were stoppered, shaken periodically, left overnight, and the pH values obtained the following day on the Beckman pH meter. All data are in terms of average pH and not average hydrogen ion concentration values converted to pH values.

Analysis of the data shows many significant differences between treatments. The main effects indicate that the difference between controls and soil-charcoal mixtures is highly significant. The pH of the controls is 5.15, whereas that of the charcoal mixtures is 5.85. Hence charcoal decreases the acidity of the soil. A highly significant difference also exists between the mixtures containing hardwood charcoal and those with conifer charcoal, the former having a pH of 6.15 and the latter of 5.55. Thus, the hardwood charcoal is more effective in increasing the alkalinity of the soil than the conifer charcoal. Also, highly significant differences exist between soils with fine charcoal and soils with coarse charcoal, the treatments containing fine charcoal having a pH of 6.21 and those containing coarse charcoal a pH of 5.49. Fine charcoal is, therefore, more effective than the coarse in increasing pH.

A positive highly significant linear trend in pH is indicated by the addition of charcoal in 15, 30, and 45 percent amounts, the values being pH 5.61, 5.84, and 6.10, respectively, thus increasing the alkalinity directly with the amount of charcoal added to the soil. The soil series also differ from one another in a highly significant manner. The pH values for these soil series are 5.78, 5.65, and 5.97 for the sand, loam, and clay series, respectively.

Many of the first order interactions are also significant. The interaction, type \times size, proves highly significant, the values being presented in Table 13.

TABLE 13. Interaction of type \times size of pH values.

Type of charcoal	SIZE OF CHARCOAL	
	< 1mm.	2-5mm.
Hardwood.....	6.63	5.67
Coifer.....	5.79	5.32

Thus, the increase in pH in treatments containing fine hardwood charcoal over those containing coarse is greater than the increase in pH in treatments containing fine conifer charcoal over those containing coarse charcoal.

The interaction, type \times [(loam + sand) vs. clay] is also highly significant. The values are presented in Table 14. In both cases treatments containing

TABLE 14. Interaction of type \times [(loam + sand) vs. clay] of pH values.

Soil	TYPE OF CHARCOAL	
	Hardwood	Conifer
Loam + sand.....	6.14	5.42
Clay.....	6.17	5.83

hardwood charcoal have higher pH values than those containing conifer charcoal, but the difference between them is greater when the charcoal is mixed with loam and sand than with clay.

Similar results are also obtained for the interaction, size \times [(loam + sand) vs. clay], which is highly significant, and presented in Table 15. Treatments

TABLE 15. Interaction of size \times [(loam + sand) vs. clay] of pH values.

Soil	SIZE OF CHARCOAL	
	< 1mm.	2-5mm.
Loam + sand.....	6.20	5.36
Clay.....	6.23	5.76

containing fine charcoal have higher pH values for both soils, but a greater difference exists between sizes in the loam and sand.

Three linear relationships which are highly significant indicate that:

1. pH is increased to a greater extent by the addition of charcoal to the loam and sand than to clay.
2. pH of the soils is increased to a greater extent by the addition of hardwood charcoal than of conifer charcoal.
3. pH of the soils is increased to a greater extent by the addition of fine charcoal than of coarse charcoal.

The analysis proves linear regression highly significant when all soil-charcoal mixtures are grouped and the controls included. The regression line is presented in Figure 7.

The difference in pH between the charcoal is due to the ash content. The hardwood charcoal which causes the greatest increase in pH when added to the soil has an ash content of 6.38 percent, whereas the conifer charcoal has an ash content of only 1.48 percent. Fine charcoal causes a greater change in an alkaline direction than does the coarse. This difference is probably due to a greater surface area of fine

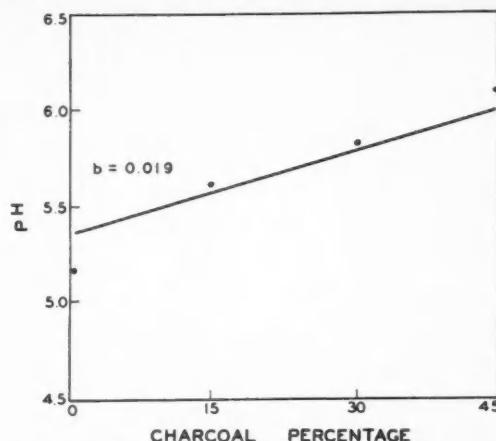


FIG. 7. Effect of charcoal on pH of soil. Types and size classes of charcoal are grouped by percentage.

charcoal than of the coarse being exposed to the soil solution; the ash can be more readily dissolved from the increased surface area.

Calcium, magnesium, and potassium are the most common bases present in the ash of wood. These elements occur in compounds that are for the most part readily soluble. When charcoal is moistened, these soluble substances are dissolved and washed out of the carbon framework of the charcoal into the soil solution. When the salts of these bases go into solution they undergo hydrolysis and develop alkalinity (Lyon and Buckman 1943: 289).

All significant first order interactions which include (loam + sand) vs. clay, as shown above, indicate that pH values of clay are less affected by addition of charcoal than are those of loam and sand. Charcoal increases the pH of sand and loam to a greater extent than it increases the pH of clay because of differences in buffering. Clay is more highly buffered than light-textured soils, especially when considered on a weight basis, and the basic ions added to the clay by the ash of the charcoal have less effect on the reaction of the soil than when added to sand. Hence, the increase in pH with the addition of equal amounts of charcoal (and its ash) is proportionally greater in the sand than in the clay. This is in agreement with a fact known by agriculturalists for some time, that more lime is needed to raise the pH of a highly buffered soil than of a poorly buffered one. Lyon and Buckman (1943: 367) present a table showing that higher amounts of lime need to be added to a clay loam than to a sandy loam to accomplish the same results.

A similar situation exists when charcoal is added to sand and loam. The sand has a pH of 4.90 and the loam a pH of 4.92, thus the soil reaction of the two is essentially the same. The sand series has a pH of 5.78 and the loam series has a pH of 5.65. The treatments containing charcoal have been included in these two series, and it may be noticed that the

loam series has a lower pH than does the sand series. This also is due to differences in buffering between the two soils. Loam has a higher buffer capacity than sand, which prevents an increase in an alkaline direction equal to that obtained for the sand when charcoal is added.

EFFECT OF CHARCOAL ON ANTIACID BUFFER CAPACITY OF SOILS

Buffer capacity of sand and sand-charcoal mixtures containing 45 percent charcoal, on a volume basis, was measured to determine the effect of charcoal on the antiacid buffer capacity of sand. Conifer and hardwood charcoal in sizes less than 1 mm. and 2 to 5 mm. were tested, making a total of four tests.

Buffer capacity was tested as follows: Five-gram samples of sand or sand-charcoal mixtures were placed in each of nine flasks. Distilled water, free of carbon dioxide, and 0.1 normal hydrochloric acid were added to produce a series containing 0.0, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 1.5, and 2.0 milligram equivalents of hydrogen ions. The total volume of the liquid added was 100 cc. per flask. The flasks were stoppered, shaken occasionally over a 24-hour period, and the pH determined with the Beckman pH meter. The concentration of hydrogen ions in the solution was determined directly from the pH value obtained (Clark 1928: 673).

The resulting buffer curves are presented in Figure 8 and are based on volume of buffering material. Conifer charcoal less than 1 mm. and 2 to 5 mm., and hardwood charcoal 2 to 5 mm. in diameter lower the buffer capacity of the sand, although the latter is slightly more active than the sand when small amounts of hydrogen ions are added. Hardwood charcoal particles less than 1 mm. in diameter increase the buffer capacity of sand.

A triplicate series of sand, loam, and clay, and combinations of them with the two types and sizes of charcoal, and totaling 39 treatments were tested to determine the effect of charcoal on buffer capacity of the soils with the addition of 0.6 and 1.0 milligram equivalents of hydrogen.

Where 0.6 milligram equivalents of hydrogen ions are added the general effect of the charcoal is to reduce the buffer capacity, as the soils have a capacity of 78.62 percent as compared with 73.94 percent for the treatments containing charcoal. Treatments containing conifer charcoal have a lower buffer capacity than do those with hardwood charcoal, 68.14 percent as compared with 79.75 percent, a highly significant difference. The treatments containing fine charcoal inactivate 79.75 percent of the ions, whereas those with coarse charcoal inactivate 68.14 percent of the ions. Thus the coarse charcoal is less effective than the fine in buffering. The data indicate an increase in buffering with the addition of fine hardwood charcoal, and a reduction in buffering with the addition of the others. Both loam and clay have a higher buffer capacity than sand, but loam has a slightly higher buffer capacity than clay when compared on a

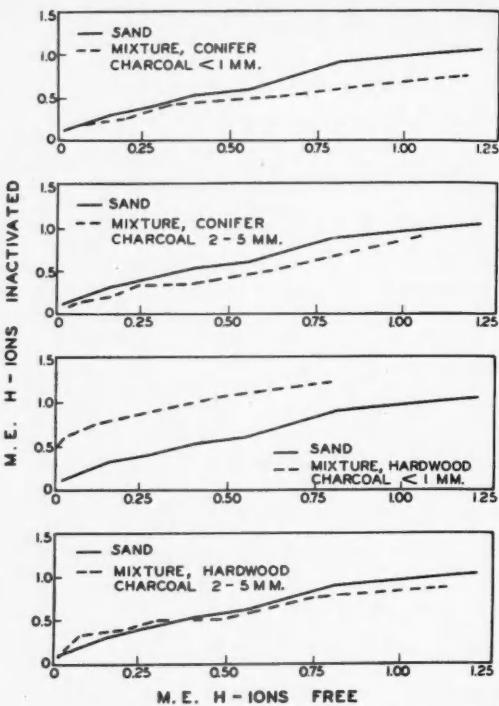


FIG. 8. Antiacid buffer capacity of sand and sand-charcoal mixtures (45 percent charcoal by volume).

volume basis. However, when compared on a weight basis the values for clay are higher, a conclusion reached by most investigators.

Several of the interactions are highly significant, and bring out principally the two following conditions:

1. The greater effectiveness of hardwood charcoal, less than 1 mm., in increasing buffering, is indicated, whereas the other charcoals cause a reduction.

2. Fine hardwood charcoal increases the buffer capacity of a sand to a greater extent than it does that of a more fine-textured soil. The buffer capacity of the loam and clay series is reduced to a greater extent than is that of the sand series. As the buffer capacities of the soils vary while those of the charcoals remain constant, these differences result, so that the higher the buffer capacity of the soil the greater will be the reduction in buffering by the addition of the coarse conifer charcoal.

A comparison of the results obtained by the analysis of the series having one milligram equivalent of hydrogen added and the series having 0.6 milligram equivalents of hydrogen added, just discussed above, indicates very close agreement between the two, and for this reason they will not be discussed. However, analysis of the data of the two series indicates a higher percentage of inactivation of the hydrogen ions with addition of smaller amounts of the acid (0.6 milligram equivalents of hydrogen).

In 1935 Jörgensen defined a buffer as a chemical system that possesses the ability to maintain a constant hydrogen ion concentration in the presence of added acids or bases (Wiegner and Pallman 1938: 170). While charcoal does not have a high antiacid buffer capacity, it is active to some extent. Undoubtedly the salts of weak acids and strong bases contained in the ash are largely responsible for the action. Hence, the higher the percentage of ash for particles of equal size the greater should be the buffer capacity, as more area is exposed and better able to release materials which act as a buffer. Evidently base exchange is also increased with the decrease in size of the charcoal particles and presumably this exchange will contribute somewhat to the capacity to buffer; however, no information on this latter subject has been obtained. The data presented show that the higher the ash content of charcoal, and the smaller the size of the particles, the greater the buffer capacity. The soils containing hardwood charcoal are better buffered than those with conifer charcoal, and soils containing fine charcoal inactivate a higher percentage of hydrogen ions than those containing coarse charcoal.

In the treatment above only antiacid buffer capacity, or the capacity to resist a change to a more acid condition, has been considered as the reaction of forest soils tends to become acid rather than more alkaline due to ecological changes. Such factors as production of acids by microorganisms, leaching of bases, and replacement of bases by hydrogen ions as a result of contact exchange between plant roots and soil colloids tend to bring about a more acid condition of the soil. Because of this tendency of a forest soil to become more acid, a high capacity to buffer against a change toward an acid reaction is considered beneficial. However, Bovie (1915) studied the ability of animal charcoal to buffer against a change to an alkaline as well as to an acid condition, and found that the charcoal tested would buffer to some extent against a change toward either an acid or basic condition.

EFFECT OF ASH CONTENT OF CHARCOAL ON ANTIACID BUFFER CAPACITY

Ash content of charcoal was reduced and tests made of the treated charcoal to determine the effect of reduction of ash on buffer relations. Fine hardwood charcoal was selected, as it had a higher ash content and buffer capacity than conifer charcoal. Reduction of ash in the charcoal was accomplished by two methods, which produced charcoals with different percentages of ash. The first method involved boiling the charcoal thoroughly in distilled water and filtering. This process was repeated five times, after which the charcoal was dried and then tested. The second method involved boiling the charcoal in 2N hydrochloric acid, soaking, washing with distilled water, and filtering six times. The ash content was deter-

mined for the regular and treated charcoal by combustion in a muffle furnace, and correcting for amount of sand in the sample according to the method outlined by the Association of Official Agricultural Chemists (1940: 125).

The ash content of the untreated hardwood charcoal was 6.38 percent, that of the charcoal boiled in water 4.90 percent, and that boiled in hydrochloric acid 1.07 percent. The buffer capacity was measured in the same manner as described in the above section, although only six flasks were used in each series. The amounts of hydrogen ions added were 0.0, 1.0, 2.0, 4.0, 6.0, and 8.0 milligram equivalents. The results are presented in Figure 9, curves A, B, and C representing the results obtained from the non-treated charcoal, charcoal boiled in distilled water, and charcoal boiled in hydrochloric acid, respectively. All data are based on a weight rather than volume relationship, as volume-weights are about equal and either basis is suitable for comparison.

The charcoal with the lowest percentage of ash has the lowest capacity for buffering, and that with the highest ash content the greatest capacity for buffering. The greatest difference in buffering between the three charcoals occurs when small amounts of acid are added. Hence, the ash content of charcoal is an important factor in determining the capacity of a charcoal to buffer against a change to a more acid condition, and if the percentage of ash is reduced the effectiveness of the charcoal to act as a buffer is also reduced. The discussion of differences in buffering in the section, "Effect of Charcoal on Antiacid Buffer Capacity of Soils" pointed out that the lower buffer capacity of conifer charcoal compared to hardwood charcoal was probably due to the lower percentage of ash of the conifer charcoal. Such a conclusion appears justified as reducing the percentage of ash of charcoal reduces its capacity to buffer.

COMPARISON OF ANTIACID BUFFER CAPACITY OF CHARCOAL AND CERTAIN LEAVES

Leaves of three species of eastern trees, collected in mid-July, were tested to determine their antiacid buffer capacity and the results were compared with those obtained for fine hardwood and conifer charcoal. The three species of leaves tested were from eastern white pine (*Pinus strobus* L.), red maple (*Acer rubrum* L.), and white ash (*Fraxinus americana* L.). The method of testing the capacity of the leaves to buffer against a change to a more acid condition was exactly the same as has been described. A weight basis was used for all samples.

The curves of antiacid buffering for the leaves and charcoals (Figure 10) indicate that both fine conifer and hardwood charcoal have a lower capacity to buffer than do any of the leaves. However, pine needles have a lesser capacity to buffer than fine hardwood charcoal when small amounts of hydrogen are added.

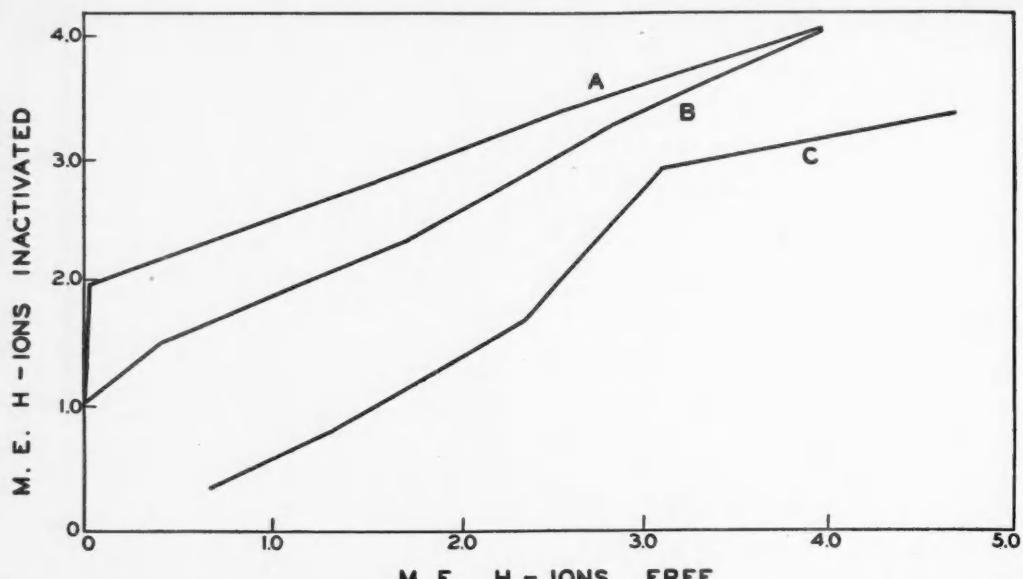


FIG. 9. Effect of ash content of charcoal on antiacid buffer capacity of fine hardwood charcoal. A. Not treated and has an ash content of 6.38 percent. B. Boiled in water and has an ash content of 4.90 percent. C. Boiled in hydrochloric acid and has an ash content of 1.07 percent.

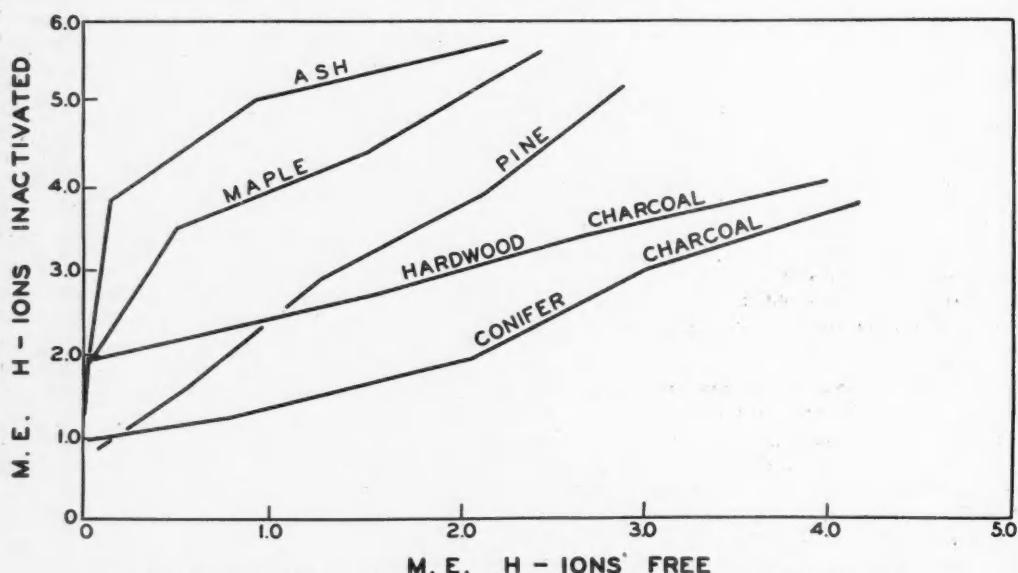


FIG. 10. Antiacid buffer capacity of charcoal compared to that of leaves of some forest trees.

EFFECT OF CHARCOAL ON AMOUNT OF AVAILABLE NUTRIENTS AND BASE EXCHANGE PROPERTIES

The more important available nutrients and base exchange capacity of the soils in pine-seedling cultures of soils and charcoals were studied to determine the chemical changes in the soil brought about by the addition of charcoal. This study took place during

the second growing season, two months before the seedlings were removed for measurements of growth. Only the controls and the soils containing fine charcoal were tested, as the coarse charcoal would be lost in sieving preliminary to testing and obviously the results would be incorrect. If, however, the soils containing the large particles were ground to pass through a fine sieve, a mixture essentially the same

as that being tested would result and the data obtained would not apply to the mixtures of soils and coarse charcoal. The soils of the seedling cultures tested are discussed under the heading, "Effect of Charcoal on Biological Properties of Two Forest Soils."

Each sample was a composite of at least three subsamples collected in the upper inch of soil. The information obtained from tests of available nutrients and base exchange capacity is presented on a weight basis in Table 16 and on a volume basis in Table 17. The data were not analyzed statistically, as nearly all changes in the chemical properties of the soil brought about by the addition of the charcoal were apparent by inspection of the data.

The term "available" is applied to amounts of calcium and magnesium rather than the more common term, "exchangeable." The total of exchangeable ions cannot exceed the base exchange capacity, and it will be noticed that with the addition of charcoal to the soil calcium alone may exceed this exchange capacity. From Table 17 it may be observed that the amount of calcium measured in the treatments containing 45 percent hardwood charcoal is about three times as great as the base exchange capacity. Much of the calcium and other bases in the ash of charcoal must exist in the carbon framework in a rather free condition, and may be leached out by solutions without an exchange of ions. For such a condition, "available" rather than "exchangeable" seems to be a better term. In order to be consistent with other data already presented, only the chemical changes in equal volumes of the soils and mixtures (Table 17) will be discussed.

BASE EXCHANGE CAPACITY

The capacity for base exchange was tested by methods outlined by Peech (1941). Hardwood char-

TABLE 16. Base exchange capacity and content of nutrients in soils of cultures of seedlings (weight basis).

Soil	Treatment		Base exch. capacity m.e. per 100 gm. soil	Percentage total nitrogen	Available P ₂ O ₅ Parts per million	Available K ₂ O m.e. per 100 gm. soil	Available Ca m.e. per 100 gm. soil	Available Mg
	Type	Charcoal Percentage						
SAND	Hard-wood	0	3.4	0.07	16.0	12.4	1.00	.17
		15	4.2	.12	52.7	104.0	6.01	.29
		30	5.1	.24	85.7	216.9	13.46	.41
		45	5.9	.26	86.3	266.4	18.56	.71
	Conifer	15	3.0	.08	16.1	55.3	1.10	.11
		30	3.3	.07	36.8	110.7	2.26	.30
		45	3.3	.08	39.3	105.2	2.80	.36
	Loam	0	4.4	.06	8.2	74.9	1.78	.38
		15	5.4	.06	15.0	176.5	6.16	.36
		30	6.6	.19	44.1	280.9	12.80	.56
		45	6.9	.25	61.8	384.9	19.81	.74
	Conifer	15	4.3	.06	10.2	102.5	1.82	.33
		30	4.3	.07	13.0	135.7	3.03	.52
		45	4.2	.07	23.7	172.7	4.90	.62

TABLE 17. Base exchange capacity and content of nutrients in soils of cultures of seedlings (volume basis).

Soil	Treatment		Base exch. capacity m.e. per 100 cc. soil	Percentage total nitrogen	Available P ₂ O ₅ gm. per million cc.	Available K ₂ O	Available Ca m.e. per 100 cc. soil	Available Mg
	Type	Charcoal Percentage						
SAND	Hard-wood	0	4.5	.09	21.4	16.6	1.34	.23
		15	5.2	.15	65.7	129.7	7.49	.37
		30	5.8	.28	98.1	248.2	15.41	.47
		45	6.1	.27	89.2	275.2	19.17	.73
	Conifer	15	3.6	.10	19.6	67.2	1.34	.14
		30	3.6	.08	40.4	121.5	2.48	.47
		45	3.1	.08	37.7	100.9	2.77	.34
	Loam	0	5.3	.07	9.9	90.5	2.15	.46
		15	6.2	.07	17.1	201.5	7.03	.41
		30	7.1	.20	47.5	293.2	13.81	.60
		45	6.9	.25	62.3	387.9	19.96	.74
	Conifer	15	4.8	.07	11.4	114.6	2.04	.37
		30	4.4	.07	13.3	139.8	3.12	.54
		45	3.9	.06	19.2	160.5	4.56	.58

coal increases the exchange capacity substantially, but conifer charcoal causes a reduction.

PERCENTAGE OF TOTAL NITROGEN

Total nitrogen was tested by the Kjeldahl method. All forms of nitrogen with the exception of nitrate nitrogen are determined by this method. Hardwood charcoal increases the nitrogen content of the soils, but a slight decrease in total nitrogen occurs with the addition of conifer charcoal. The reason for the higher nitrogen content of the hardwood charcoal is not understood, and as the percentage of ash was considerably higher than that of the conifer charcoal, the temperature during the charring process must have been at least as high. The high temperature necessary for charring should have driven off most of the nitrogen in the form of gas so that little remained.

The content of total nitrogen of the original unused materials, on a volume basis, are as follows:

Percent

Sand	0.09
Loam	0.08
Hardwood charcoal	0.21
Conifer charcoal	0.01

Therefore it is to be expected that the cultures containing hardwood charcoal would have a higher content of total nitrogen than the control, but that those containing conifer charcoal would be lower.

AVAILABLE NITROGEN

Ammoniacal and nitrate nitrogen are available to plants. The content of available nitrogen was tested by the methods of Noggle and Wynd (1941). Only a trace of ammoniacal and nitrate nitrogen could be detected in the soils and soil-charcoal mixtures tested, including the treatments containing a high per-

centage of fine hardwood charcoal which has a high content of total nitrogen. As only a trace could be detected, the results are not presented. Apparently the nitrogen was used by the seedlings as soon as it became available.

AVAILABLE PHOSPHORUS

The availability of phosphorus was tested by the method of Truog (1930). Both hardwood and conifer charcoal increase the amount of phosphorus available in the soil, although a larger amount is added to the soil by the hardwood charcoal.

AVAILABLE POTASH

The determination of available potash (Volk and Truog 1934) indicates a large increase of potash added to the soil by addition of charcoal. The hardwood charcoal is especially active in this respect, as a 45 percent mixture with loam increases the available K_2O of the loam from 90.5 to 387.9 gm. per cc., and a 45 percent mixture with sand increases the available K_2O of the sand from 16.6 to 275.2 gm. per cc.

AVAILABLE CALCIUM

Available calcium was determined by the method of Wilde (1942: 186). Both charcoals increase the percentage of available calcium in the soil, but here again the hardwood charcoal is especially active, increasing the content about 15 times when 45 percent by volume is added to the sand, and about 10 times when 45 percent by volume is added to the loam.

AVAILABLE MAGNESIUM

Available magnesium was determined by the method of Wilde (1942: 188). An increase in amount of available magnesium is indicated with the addition of either hardwood or conifer charcoal to the soil. Hardwood charcoal adds more available magnesium than the conifer. The rate of increase is less than that found for available calcium.

Probably the small amounts of charcoal normally found in forest soils improve the chemical relations by the addition of nutrient ions, and the decrease in hydrogen ion concentration will favor nitrification. However, large amounts of charcoal such as are found in localized areas, as charcoal hearths, may add ions to the soil solutions to such an extent that they will be toxic to the vegetation.

EFFECT OF CHARCOAL ON BIOLOGICAL PROPERTIES OF TWO FOREST SOILS

Crocks of soil and charcoal mixtures were set up in a greenhouse and sown with eastern white pine seed and measurements taken to determine the effects of charcoal in the soil on germination of the seed, growth of the seedlings, and numbers of fungi and heterotrophic, aerobic bacteria and actinomycetes. Effect of charcoal on mycorrhizal abundance and damping-off was also observed.

ESTABLISHMENT OF CULTURES

A duplicate series of 26 crocks was set up with two soils and mixtures of the soils and charcoals in a

randomized manner. The soils were sand and loam; the charcoals were hardwood and conifer, in less than 1 mm. and 2 to 5 mm. sizes, and the amounts of charcoal used were 15, 30, and 45 percent on a volume basis.

The soils and mixtures were placed in 2-gallon glazed crocks having an opening in the bottom for drainage. These were then filled to a depth of about 2.5 inches with either sand or loam, and the rest of the crock, about 3.5 inches, was filled with the soil-charcoal mixture. This was believed to simulate more nearly conditions where charcoal appeared in soils in nature or when added to forest nursery soils than would a complete filling of the crocks with the soil-charcoal mixture.

The crocks were filled with the soils and mixtures May 30 and 31, 1943, and June 12 white pine seeds were sown and covered with the same material as that in the crock. Moisture conditions were kept as nearly optimum as possible for germination of the seed and growth of the seedlings by daily examination of the soils or mixtures in the individual crocks during the first season and watering when necessary. Each crock with its medium and seedlings is called a "culture" or "seedling culture."

Germination counts were made daily the first 45 days following sowing and after that by 5-day periods. Seedling loss by damping-off was recorded throughout the summer and autumn.

At the end of the first growing season the shoots of all but 20 seedlings were cut at ground level, and in April of the following spring the shoots of all but 10 seedlings were cut at ground level, thus allowing 10 seedlings per culture for growth studies. In both instances the seedlings removed were those with the greatest growth deviation from the average and those most densely packed. The reduction of seedlings in the different cultures to an equal number allowed approximately an equal area from which each seedling could draw its nutrients and moisture, thus eliminating growth differences due to variations in density of stocking.

GERMINATION RESULTS

The percentages of germination were analyzed for both the 60- and 360-day germination periods. Both analyses gave significant differences between treatments containing fine and coarse charcoal, and between those with different percentages of charcoal which give a linear relationship. Table 18 summarizes the data where significant differences exist, showing that germination is reduced with the increase of charcoal in the soil and that fine charcoal is more effective in reducing germination than the coarse.

Germination trends for a period of 100 days after sowing are shown in Figures 11 and 12 for the treatments having a real effect on germination, that is, percentage of charcoal and size of charcoal particles. These curves show that the differences brought out by the analyses continue in a uniform manner throughout the first 100 days.

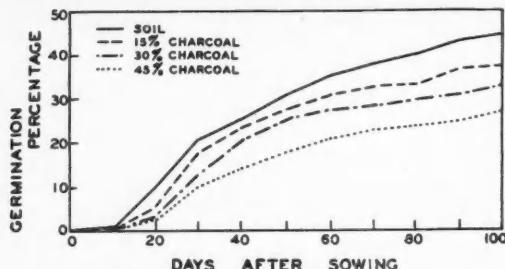


FIG. 11. Effect of charcoal particles, <1 mm., in the soil on germination of white pine seed.

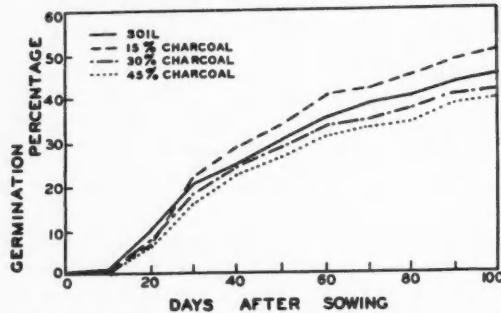


FIG. 12. Effect of charcoal particles, 2-5 mm., in the soil on germination of white pine seed.

TABLE 18. Influence of amount and size of charcoal on germination of white pine seed during two periods.

Period	GERMINATION PERCENTAGE						
	Percentage of charcoal				Size of charcoal		
	0	15	30	45	<1mm.	2-5mm.	
60 days.....	35.6	35.7	30.3	26.1	26.2	35.1	
360 days.....	51.5	54.0	47.1	42.8	41.3	54.6	

An analysis of variance was employed to test the rate of change of germination for both the 60-day and 360-day periods with the addition of charcoal in increasing linear amounts. In using this method the different types and sizes of charcoal were grouped together so that a general effect of the charcoal was obtained. This method also brings out any effect that the check (sand and loam) may have on the regression, information not supplied by the former tests. The effect of the charcoals in reducing germination for the two periods is clearly shown in Figure 13.

CAUSE OF GERMINATION DIFFERENCES

No attempt was made to determine by experimentation the properties of charcoal responsible for reduction in germination percentage of pine seed. However, with the information previously given in this paper on physical and chemical properties of soil-charcoal mixtures and information supplied by various authors on external factors influencing ger-

mination it is possible to suggest how charcoal affects germination.

The best treatment to date of the effect of environmental factors on forest tree seed germination is that of Baldwin (1942: 118). He lists temperature, moisture supply, external oxygen supply, carbon dioxide, light, atmospheric pressure, depth of covering, acidity and alkalinity of substratum, and toxicity of substratum as influencing germination and discusses each briefly. In addition to these factors numbers of micro-organisms may be considered, as saprophytic fungi attack the seed coats and may hasten germination.

Most of these factors appear to be about equal in all the cultures with the exception of acidity and alkalinity of substratum and toxicity of substratum. It is doubtful if the changes of the physical properties brought about by the addition of charcoal would hinder germination, especially under greenhouse conditions. In the section, "Effect of Charcoal on Physical Properties of Three Forest Soils" it was shown that only slight changes occur in the physical properties of loam when charcoal is added. As the reduction in germination with the addition of charcoal to loam was about the same as for sand, whose physical properties are changed by the addition of charcoal, it appears that other factors than changes in physical properties are responsible for the differences in germination. Differences in germination cannot be due to soil microorganisms as they were found to be present in all cultures in nearly equal amounts.

The study of pH values and available nutrients shows that charcoal increases both pH and the amount

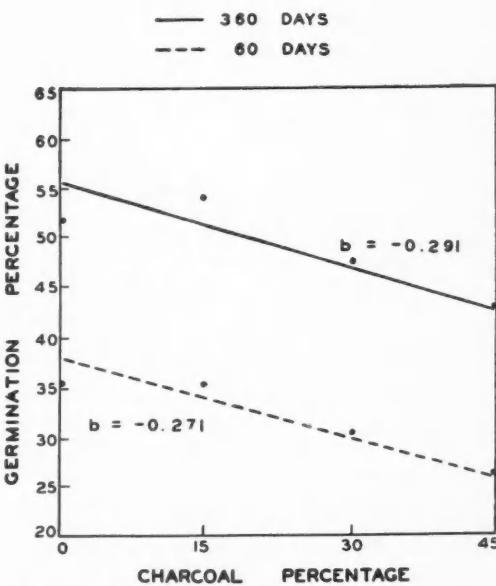


FIG. 13. Effect of charcoal on germination percentage of white pine seed at 60 and 360 days. Types and size classes of charcoal are grouped by percentage.

of available nutrients, and both factors are believed to influence germination.

Studies of the effect of hydrogen ion concentration on the germination of seed have not given wholly conclusive results although most of the experiments seem to indicate that a slightly acid substratum (pH 5.5 to 6.5) is most favorable. Baker (1934: 137) expressed the view that acidity, in the degree developed in forest soils, acts as a stimulant to germination and gave pH values 5.4 to 6.0 as the reaction most suitable for germination of Scotch pine (*Pinus sylvestris* L.). Salter and McIlvaine (1920) found the optimum reaction for the germination of certain cereal seed above pH 2.96 and below pH 7.71. In contrast to this, Fisher (1935) tested conifer seed on different substrata at the hydrogen ion concentrations of pH 5.96 to 7.66 and concluded that the hydrogen ion concentration of surface material had no direct correlation with either total germination percentage or individual species germination percentages. Similar conclusions were reached by Thrupp (1938), who found, by adjusting reaction with sulfuric acid and calcium hydroxide, that conifer seed, including western white pine (*Pinus monticola* D. Don.), germinates well over a wide range of hydrogen ion concentration; he reported germination at pH values as high as 10.

Germination tests in wood ashes have also given conflicting results. Fabrius (1929) found that the ash was detrimental to germination, whereas Eneroth (1931) found no inhibiting effect.

From the above information it seems doubtful if the hydrogen ion concentrations in the soil-charcoal cultures, which vary only from pH 4.9 to 7.2, would cause real differences in germination, as most investigators found a greater range of hydrogen ion concentration necessary than was obtained in any of the cultures before germination of forest tree seed is affected.

Toxicity of chemical substances to germination of seeds has been shown experimentally by Sayer and Clark (1935) and Hicks (1900), the latter believing that the injury is caused by plasmolysis as a result of high concentration of soluble salts. He stated, "the chief injury to germination from chemical fertilizers is inflicted upon the young sprouts after they leave the seed coat and before they emerge from the soil, while the seeds themselves are injured only slightly or not at all." Also, it is known that salt solutions of high concentrations decrease the capacity of seeds to absorb water (Rudolfs 1921). Schmidt (1929) found that the accumulation of salts on burned areas made absorption of water by seeds difficult and reduced germination percentage. If salts from the charcoals were present in sufficient quantities it seems very probable that they could reduce germination in the soil-charcoal cultures.

An examination of Table 16 indicates that available calcium, potassium, magnesium, and phosphorus in the cultures were increased several times with the addition of charcoal. This is especially true of potassium, which increases from 12.4 to 266.4 parts

per million in the sand-hardwood charcoal cultures, and from 12.4 to 105.2 parts per million in the sand-conifer charcoal cultures. The suggestion is made that the higher concentrations of the soil solution resulting from the soluble ash of the charcoal is the cause of the differences in germination. As none of the germinating seeds were observed to possess plasmolized tissues, it seems probable that the higher concentration of the soil solutions where charcoal was present decreased water absorption by the seed which presumably caused a reduction in germination percentage.

The greater amounts of charcoal added and the fine charcoal decreased germination the most, and in both instances the amount of soluble chemical substances present was high. The coarse charcoal, 15 percent mixture, is shown in Figure 12 as actually increasing germination, which is contrary to the other results. In this treatment only a very slight increase in the concentration of the soil solution would occur and this presumably acts as a stimulant to germination. This is in agreement with Schmidt (1929) who reported a favorable effect of weak concentrations of salts from wood ashes on germination of pine seed.

When charcoal with a high ash content occurs in the soil in nature, a greater reduction in germination than occurred in the cultures should take place, especially during dry periods, as the soil solution will become more concentrated as moisture is lost from the soil by evaporation and transpiration. Wherever large amounts of ash occur in the forest as a result of burning, even greater reduction in germination may be expected due to the high concentration of soluble salts.

GROWTH RESULTS

The ten white pine seedlings in each culture were collected in September 1944, at the end of the second growing season, by removing the soil from the crocks and shaking and washing out the roots. Shoot lengths were measured, abundance of mycorrhizae recorded, and the average seedling from each culture selected and photographed. The other nine seedlings were cut at the root crown, and the roots were washed. Shoots and roots were oven-dried and weighed separately on an analytical balance. The data obtained were total weight of seedlings, weight of shoot, weight of root, and shoot-root ratio based on oven-dry weight, and represent the average of nine seedlings per culture. All seedlings appeared healthy although during August and early September of the second growing season a yellowing of the tips of the needles became apparent. This condition was evident in some of the seedlings of all the cultures and no differences in frequency or amount could be observed between treatments, so that the cause was present in all cultures to about the same degree.

LENGTH OF SHOOT

The analysis of average length of seedling shoots shows that charcoal exerts a significant influence on height growth and that highly significant differences

exist between the treatments containing fine and coarse charcoal and between those with different percentages of charcoal which gives a linear relationship.

The shoots in the check cultures are 2.75 inches high, whereas in the cultures containing charcoal they are 2.46 inches high. The shoots in the cultures having coarse charcoal are 2.54 inches high and those in cultures with fine charcoal are only 2.37 inches high.

The cultures having charcoal in amounts of 0, 15, 30, and 45 percent have average height growth values of 2.75, 2.60, 2.44, and 2.32 inches, respectively. Thus addition of charcoal to the soil decreases height growth, fine charcoal being more effective than the coarse.

The first order interaction, type of charcoal \times linear relationship, is significant and is presented in Table 19. A real difference in linear relationship is

TABLE 19. Interaction of type \times linear relationship of height growth of white pine seedlings.

Type of charcoal	PERCENTAGE OF CHARCOAL		
	15	30	45
Hardwood	2.64	2.44	2.18
Conifer	2.57	2.44	2.47

indicated, the hardwood charcoal line being steeper than the conifer. This means that the length of the shoot is reduced to a greater extent by the addition of hardwood charcoal than by conifer charcoal to the soil.

This significant difference applies only to the differences in slope between the treatments with hardwood charcoal and those with conifer charcoal; not to the total differences in growth between treatments containing the two types of charcoal, as the over-all difference between conifer and hardwood charcoal is not significant. An analysis of variance which tests linear relationship and includes soil indicates linear regression to be highly significant. The regression line in Figure 14 shows the reduction in height growth with the addition of charcoal to the culture.

TOTAL WEIGHT OF SEEDLINGS

The analysis of average total weight of seedlings indicates no significant differences between treatments, hence the difference between the total weights of the seedlings in the cultures containing charcoal and those in the control are due to chance. The weights are 0.6773 grams and 0.7845 grams for the cultures with charcoal and the controls, respectively. However, the data indicate a progressive reduction in total weights of the seedlings in the cultures containing fine hardwood charcoal as the percentage of charcoal is increased. The weights of the seedlings in the cultures with 15, 30, and 45 percent charcoal are 0.7829 grams, 0.6851 grams, and 0.5120 grams, respectively. The data from which these values were obtained were

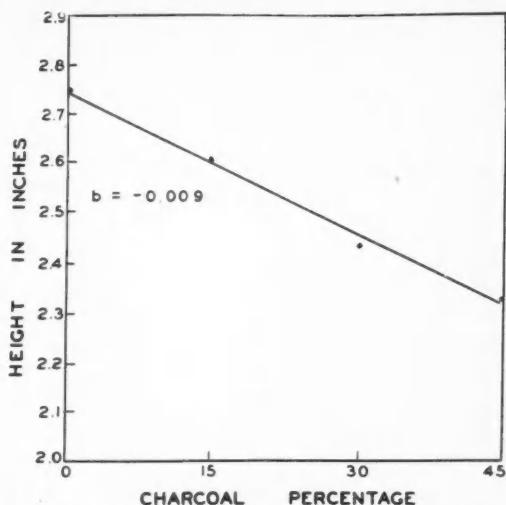


FIG. 14. Effect of charcoal on height growth of 2-0 pine seedlings. Types and size classes of charcoal are grouped by percentage.

tested by a special analysis of variance for individual degrees of freedom, and a negative linear slope proved significant. Thus the cultures containing the fine hardwood charcoal, the most active chemically, reduced the weights of the seedlings; the cultures with the highest percentage of the charcoal produced seedlings having the lowest weights. This tendency was not brought out in the regular analysis. Average seedlings of the cultures containing loam, and loam with fine hardwood charcoal, are shown in Figure 15. Average seedlings of the cultures containing sand, and sand with fine hardwood charcoal, are shown in Figure 16.

WEIGHT OF SHOOT

The analysis of average total weight of the shoots indicates no significant difference between treatments, the shoots having a weight of 0.4411 grams in the control and 0.3640 grams in the cultures containing charcoal. These data agree closely with those of total weight above. Also a reduction in weight of the shoot in the fine hardwood charcoal cultures is noticeable, being 0.4487 grams, 0.3374 grams, and 0.2626 grams for the cultures containing 15, 30, and 45 percent of fine hardwood charcoal, respectively.

WEIGHT OF ROOT

The analysis of average total weight of the roots indicates no significant difference between treatments. The difference in values of 0.3433 grams in the control and 0.3174 grams in the cultures containing charcoal is due to chance. The reduction in weight in the cultures with fine hardwood charcoal is also noticeable, but less so than in total weight of seedling or weight of shoot. The values are 0.3842 grams, 0.3477 grams, and 0.2493 grams, respectively, for the treatments containing 15, 30, and 45 percent of charcoal.

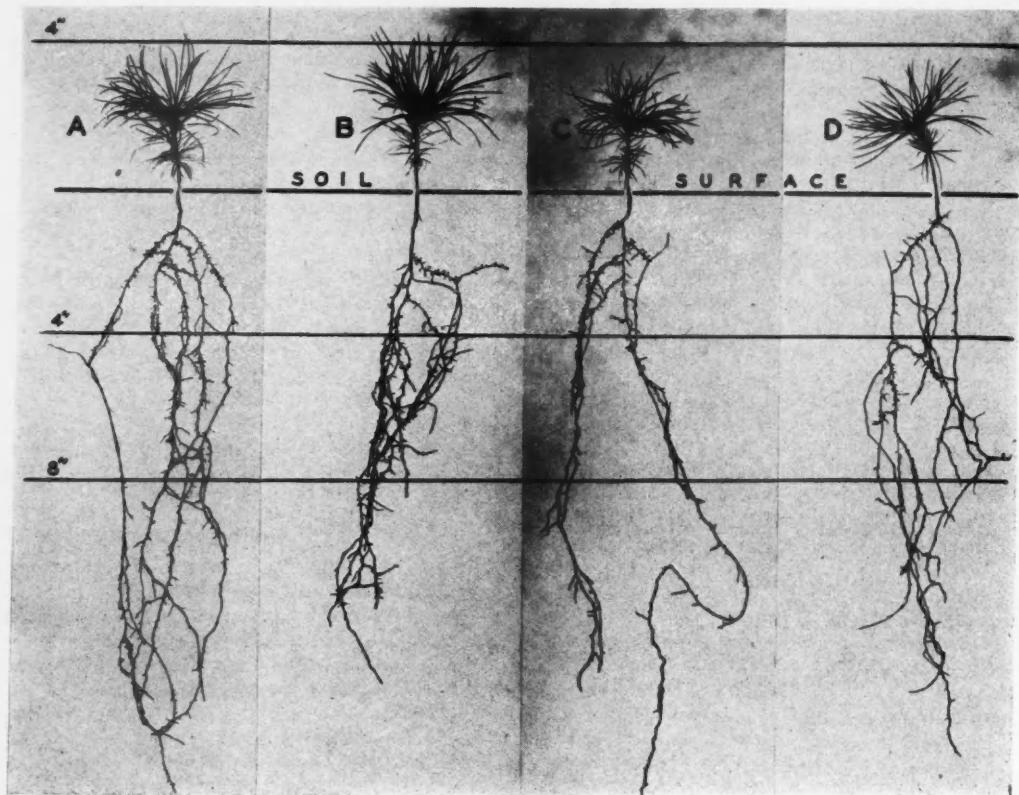


FIG. 15. Eastern white pine seedlings, 2-0 stock, grown in loam and loam mixed with fine hardwood charcoal. Each seedling represents the average of those grown in the following media:

- A. Control, loam without charcoal.
- B. Fine hardwood charcoal, 15 percent; loam 85 percent.
- C. Fine hardwood charcoal, 30 percent; loam 70 percent.
- D. Fine hardwood charcoal, 45 percent; loam 55 percent.

CAUSE OF GROWTH DIFFERENCES

The effect of addition of charcoal to the soil on growth of pine seedlings was not great; only height of shoot showed a change consistent for types and percentages of charcoal. The only difference in weight of seedlings between treatments existed in those containing fine hardwood charcoal. The causes of these changes in growth, where they exist, as for differences in germination, are probably a result of higher concentration of the soil solution caused by the high ash content of the charcoal.

Fabreius (1929) found ashes detrimental to the early growth of tree seedlings, and Moore (1922) stated that alkalinity produced by heavy applications of lime was extremely unfavorable for growth of some tree seedlings. At the state nursery, Henryville, Indiana, an accumulation of three percent calcium carbonate in the upper five inches of soil made the production of good pine seedlings impossible. The accumulation of calcium carbonate resulted from surfacing the nursery beds with calcareous sand (Anon. 1938).

The reason for height growth of the seedlings being more affected by addition of the charcoal than weight of the seedlings or weight of the shoots is not understood. Both germination and height of shoot are affected in the same manner, as the fine charcoal and increased amounts of charcoal cause a reduction in values in both tests. However, differences in height are less than differences in germination between the cultures having different amounts of charcoal. Possibly the reduction in height growth with the addition of charcoal is caused by the slower germination and resulting shorter growing season during the first year.

SHOOT-ROOT WEIGHT RATIO

The analysis of average shoot-root weight ratio for the different treatments indicates a significant difference between types of charcoal and a highly significant difference between soil series.

The seedlings in the hardwood charcoal treatments have a ratio of 1.112, whereas those in the conifer charcoal treatments have a ratio of 1.255. This indicates a somewhat heavier root system in relation to

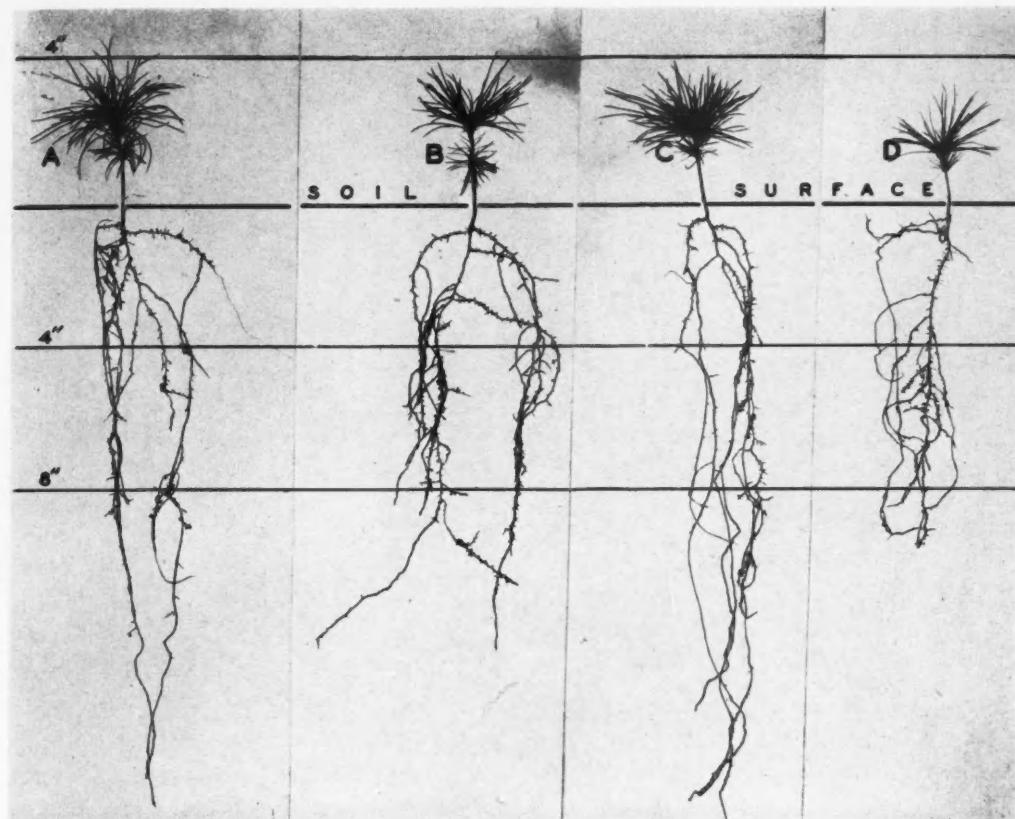


FIG. 16. Eastern white pine seedlings, 2-0 stock, grown in sand and sand mixed with fine hardwood charcoal. Each seedling represents the average of those grown in the following media:
 A. Control, sand without charcoal.
 B. Fine hardwood charcoal, 15 percent; sand 85 percent.
 C. Fine hardwood charcoal, 30 percent; sand 70 percent.
 D. Fine hardwood charcoal, 45 percent; sand 55 percent.

the shoot in the treatment containing hardwood charcoal than in those with conifer, but this difference probably means little in practical terms in spite of the statistical significance shown. The seedlings in the loam series have a ratio of 1.062, and in the sand series a ratio of 1.323, hence a heavier root system in relation to the shoot was developed in the loam series than in the sand series.

EFFECT OF CHARCOAL ON ABUNDANCE OF MYCORRHIZAE

Data were taken on abundance of mycorrhizae, tallying the number of mycorrhizae in each culture by visual inspection. The examination showed bifurcated mycorrhizae associated with the pine roots in all cultures. The method of taking the data was not considered accurate enough to warrant testing statistically the differences between treatments. However, it appeared from the examination that the addition of charcoal had no effect on abundance of mycorrhizae.

EFFECT OF CHARCOAL ON DAMPING-OFF OF WHITE PINE SEEDLINGS

Post-emergence damping-off of the white pine seedlings occurred in 38 of the 52 cultures. Actually the disease was of little importance and in no way hindered the experiment. The greatest number of seedlings lost by damping-off in any one culture was eight, and some of the cultures lost only one or two. The cultures with charcoal lost nearly four times as many seedlings as did those without charcoal, but because of the light infection it was impossible to conclude that the charcoal increased damping-off.

Under "Review of Literature," instances are cited where charcoal has been used, or recommended as a treatment, to control damping-off in the forest nursery. However, the only instance recorded where the addition of charcoal to the soil decreased damping-off was in the Mont Alto State Forest Tree Nursery in Pennsylvania (Retan 1915a). Several factors may influence the amount of damping-off (Baxter 1943:

99), the one most studied being acidity of the seed bed. As a result of numerous experiments on forest nursery soil during the past 30 years it is known that generally the best method of reducing damping-off of coniferous seedlings is by acidifying the soil.

Such substances as wood ash and lime tend to neutralize an acid soil and therefore recommendations have been made (Boyce 1938: 90; Davis, *et al.*, 1942: 52) that wood ashes should not be added to forest nursery sites. Steven (1928: 118) in a nursery fertilizer experiment found that additions of hardwood ashes to soil having a pH of 5.5 increased the pH to 6.4 and conifer wood ashes increased the pH to 7.8. Damping-off of Scotch pine and Sitka spruce was increased as a result of the changed conditions.

As the ash from the charcoal in the soil reduces the hydrogen ion concentration causing a less acid condition, it might be expected that in areas where large amounts of charcoal occurred damping-off would be increased until the ash content of the charcoal was removed by solution and leaching. Therefore, the increase in damping-off obtained in the soil-charcoal cultures might have been expected. The data showed such a tendency, but were too limited to permit definite conclusions.

NUMBER OF COLONIES OF HETEROTROPHIC, AEROBIC BACTERIA AND ACTINOMYCETES IN THE SURFACE-SOIL OF THE SEEDLING CULTURES DETERMINED BY THE PLATE METHOD

The technique used in determination of the number of colonies of heterotrophic, aerobic bacteria and actinomycetes in the surface-soil of the pine cultures by the plate method was essentially that given by Fred and Waksman (1928: 99). About 30 grams of the moist soil was obtained from each culture and placed in a sterile container. The soil was gathered from the upper inch at three different places in each culture, placed in a container, and then taken directly to the laboratory and plating-out was started.

Each sample was thoroughly mixed and diluted to 1:100,000, a value determined by preliminary tests to allow between 40 and 200 colonies per plate. Waksman (1932: 21) showed that for best results the number of colonies of bacteria and actinomycetes should be between 40 and 200 per plate, and for fungi, 20 to 100 colonies per plate for the special acid media.

One cc. of a 1:100,000 dilution sample was placed in each of seven sterile Petri dishes, and then approximately 10 cc. of sterile Bacto-Nutrient Agar (dehydrated) cooled to 40° C., was poured into each dish. The dishes were rotated to distribute the bacteria evenly and allowed to cool to room temperature, then inverted and kept in a warm room at about 27° C. for 7 days before counting the colonies.

An analysis of variance of the number of colonies on a volume basis (1 cc.) indicates that differences between treatments are not significant. Hence, the addition of charcoal has no important effect on the number of bacteria and actinomycetes when the size

classes, types, and amount are grouped together. The results of the plating show that the bacteria and actinomycetes number 6,285,000 per cc. for the soils and 6,349,583 per cc. for the soils and charcoals mixed. However, when the values of fine hardwood charcoal, the most active chemically, are plotted by percentages (Fig. 17) the addition of 45 percent charcoal tends to increase the number of these micro-organisms.

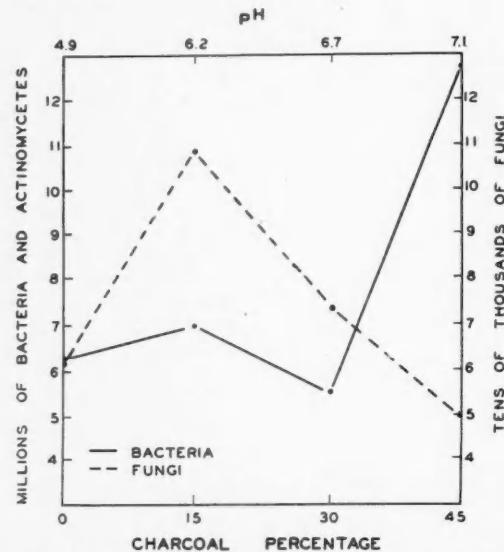


FIG. 17. Variation in numbers of colonies of bacteria and actinomycetes compared to that of fungi with addition of fine hardwood charcoal to the soil.

NUMBER OF COLONIES OF FUNGI IN THE SURFACE-SOIL OF THE SEEDLING CULTURES DETERMINED BY THE PLATE METHOD

The technique used in determining the number of fungi was similar to that of determining the number of bacteria and actinomycetes. A dilution of 1:1,000 was used, as preliminary tests showed that such a dilution gave satisfactory results.

One cc. of the 1:1,000 dilution sample was placed in each of seven sterile Petri dishes and approximately 10 cc. of sterile peptone-glucose acid agar (Waksman 1922) having a pH of 4 was melted and cooled to 40° C. and then poured into each dish. The dishes were rotated, cooled to room temperature, and kept at a temperature of about 27° C. for four days before counting.

An analysis of variance of the number of colonies of fungi on a volume basis indicates that differences between treatments are not significant. Hence, the addition of charcoal has no important effects on the number of fungi in the soils. The cultures having no charcoal give a value of 62,100 fungous colonies per cc. and those containing charcoal 76,533 colonies per cc. The data obtained for the fine hardwood charcoal were plotted (Fig. 17) and it may be ob-

served that although the cultures containing 15 percent charcoal increase the number of colonies, the 30 and 45 percent mixtures of charcoal reduce the number of colonies from the 15 percent level, the 45 percent mixture being more active in this respect than the 30 percent mixture.

ECOLOGICAL SIGNIFICANCE

The study indicates that presence of charcoal in forest soils alters several physical and chemical properties, but that these changes have little influence on the development of conifer seedlings. Much larger amounts of charcoal than normally exist in forest soils, excepting, perhaps, localized areas where charcoal hearths occur, would need to be present before a change in development of the forest type becomes of ecological importance.

The moisture relations of medium-textured soils are affected little by charcoal, but of extremely fine- and coarse-textured soils are improved. Where a coarse sand exists, available moisture may well be the limiting factor for growth during dry periods. Charcoal incorporated in a soil of this type increases the amount of available moisture and makes possible a longer period during which the plants may utilize the water. In the case of a fine clay, so much moisture may be held in the capillary pores during wet periods that the air space is reduced and not enough oxygen is present to allow normal functioning of the roots; thus the growth of the entire plant is unfavorably influenced. Charcoal incorporated in these fine-textured soils improves the moisture relations by increasing the volume of the larger capillary pores so that the field capacity is reduced. As a result better drainage occurs. Charcoal allows fine-textured soils to be more quickly drained following extremely wet periods, and the total amount of water held tends to become lower, creating better conditions for aeration.

Claims have been made in the literature that organic matter, including charcoal, changes the texture of extremely coarse and extremely fine soils. Many of the physical properties may be changed, but not texture. Soil texture is determined solely by the size-class distribution of mineral particles (Rice and Alexander 1938: 893; Lyon and Buckman 1943: 41). Organic matter is not concerned in the determination of soil textures.

Chemical changes of soil properties are brought about by incorporation of charcoal in a soil, the amount of the change depending directly upon the amount of charcoal and its ash content. Calcium and potash are increased substantially, and magnesium and phosphoric acid to a lesser extent. As available nitrogen is not increased, the ratio of available $N:P_2O_5:K_2O$ is changed. Wilde (1938; 1940) found this ratio for some conifer and hardwood forest soils to be 1:2:5 and 1:3:5. As long as ash from the charcoal remains in the soil phosphoric acid and potash will be increased, but available nitrogen present apparently will be but little affected. However, the study indicated that even when large amounts of the

charcoal caused a substantial deviation from optimum conditions, as determined by Wilde, the growth of 2-0 pine seedlings was not greatly affected. Therefore, if none of the elements essential for growth are deficient, a nearly normal rate of growth may be expected, even though the ratio of available N, P_2O_5 , and K_2O is somewhat changed from the optimum by the addition of charcoal.

There is a tendency for charcoal to improve the physical properties of a soil. The small amounts of charcoal normally found in forest soil (much less than 15 percent by volume) may improve the chemical relations by the addition of nutrient ions such as calcium, magnesium, potassium, and phosphorus. A slight decrease in soil acidity is to be expected and may favor nitrification by soil organisms, thus increasing the nitrogen available for plants. Only in extreme cases, however, are the changes in either physical or chemical properties likely to be of ecological importance.

Large amounts of charcoal (30 to 45 percent by volume) having a high ash content should never be used for improvement of the soil in nurseries growing coniferous nursery stock. The pH of the soil will be increased considerably and damping-off will be favored. The content of soluble salts may be increased to such an extent that germination of seeds is retarded and the growth of seedlings reduced.

In general, it is recommended that in forest nurseries where large amounts of organic matter are needed for improvement of the soil, materials such as peat, which is less alkaline than charcoal, be used.

SUMMARY AND CONCLUSIONS

Charcoal is believed to be a component of soils of all forest types in the United States and perhaps throughout the world. Little information is available on the effect of charcoal on soil properties and the resulting effect on vegetation. A study was undertaken to determine changes occurring in physical, chemical, and biological properties of soils as a result of addition of charcoal. Soils and charcoals were obtained and tests made from mixtures prepared in the laboratory. The three soils used in the study were Merrimac sand, Maltby sandy loam, and Berlin clay loam, all belonging to the brown podzolic group. They are referred to as sand, loam, and clay, respectively. Two types of charcoal, hardwood and conifer, in two sizes, less than 1 mm. and 2 to 5 mm., were used. The soils and charcoals were mixed so that the charcoal composed 15, 30, and 45 percent of the total by volume. Wherever possible the data have been presented on a volume rather than a weight basis. This method of presentation is believed to indicate actual conditions better than the commonly used weight basis, as volume weights differ so greatly between the soils and charcoals. From the results of the investigation the following conclusions have been reached:

1. Charcoal increased the moisture equivalent of sand, slightly increased the moisture equivalent of

loam, and decreased that of the clay. The change with the amount of charcoal added was linear for all three soils. Fine charcoal caused higher moisture equivalent values than coarse charcoal. As moisture equivalent approximates field capacity a similar change in the latter constant will occur with addition of charcoal. The moisture content at field capacity will be increased in a coarse-textured soil, reduced in a fine-textured soil, and but slightly affected in a medium-textured soil.

2. The hygroscopic coefficient was increased in sand, slightly increased in loam, and reduced in clay with the addition of charcoal. The change in hygroscopic coefficient with the addition of charcoal was linear for all three soils. Fine charcoal caused higher hygroscopic coefficients than coarse charcoal, and hardwood charcoal higher values than conifer when added to sand and loam.

3. The wilting percentage of sand was increased, that of loam but slightly increased, and that of clay decreased with addition of charcoal. The change with addition of charcoal was linear for the three soils.

No differences in wilting percentage existed between treatments containing fine and coarse charcoal in either the sand or loam series. However, the wilting percentage of clay was greater when it contained coarse charcoal than when it contained fine. Therefore, a greater amount of moisture was rendered unavailable by the large particles of charcoal than by the small when compared with the adsorptive ability of the two sizes of charcoal.

4. Addition of charcoal to the sand, loam, and clay had no effect on ratios of moisture equivalent to wilting percentage or hygroscopic coefficient to wilting percentage. Both ratios were higher for the treatments containing fine charcoal than those containing coarse charcoal, for all three soils. This again emphasizes the fact that more moisture is held by the coarse charcoal particles when permanent wilting occurs than is held by the fine charcoal particles, especially in relation to comparative amounts of moisture adsorbed.

5. Moisture available to plants was increased in the sand, not affected in the loam, and decreased in the clay by the addition of charcoal. When charcoal made up 45 percent of the mixture by volume, the available moisture of the sand was increased about 18 percent, and the available moisture of the clay decreased about 20 percent. Ecologically, such a change may be considered favorable.

6. Addition of charcoal to the soils reduced the rate of evaporation. Charcoal when added to clay was less effective in reducing evaporation than when added to the sand and loam.

7. The reduction in evaporation from the soils by addition of charcoal tended to increase the time required to reach the wilting percentage. Loss of moisture as a result of transpiration of plants was not considered in the determination.

8. Charcoal, when added to forest soils, reduced the hydrogen ion concentration causing an increase in

pH. Charcoals having a high ash content caused a greater increase in pH than did those having a low ash content. Likewise, finely ground charcoal increased the pH of soils more than coarse charcoal particles, as the ash probably was more readily dissolved from the greater surface area of the charcoal by the soil solution. However, changes in pH and other chemical properties of a soil caused by addition of charcoal may be expected to persist over a longer period of time if the particles are coarse than if they are fine, even though the changes are not so great. This may be explained by the fact that the ash is dissolved more slowly from the coarse particles than from the fine.

9. The pH of a poorly buffered soil, such as a coarse sand, is increased to a greater extent by the addition of charcoal than is that of a well buffered soil such as a fine loam or a clay.

10. A finely ground charcoal having a high ash content, when added to a soil, increased the antiacid buffer capacity of that soil to some extent. It is more effective when added to a sand than when added to a clay. The buffer capacity of soils, even a coarse sand, was reduced by the addition of charcoal having a low ash content regardless of the size of the particles, and by coarse charcoal particles even though they have a high ash content.

11. The antiacid buffer capacity of a charcoal is largely dependent upon its ash content. The higher the ash content, the greater will be its ability to buffer, providing the particles are the same size, so that equal surface areas are exposed to the solution. The buffer capacity of a charcoal may be reduced by removing its ash.

12. Charcoal is less effective as a buffer than leaves of forest trees. Even fine charcoal having a high ash content has a lower capacity to buffer than leaves of white ash, red maple or eastern white pine.

13. Fine hardwood charcoal increased base exchange capacity of soil; fine conifer charcoal slightly decreased this capacity.

14. Total nitrogen was increased by addition of the hardwood charcoal to soils; it was slightly decreased by the addition of conifer charcoal, a direct result of the content of nitrogen of the charcoal.

15. Phosphoric acid, potash, calcium, and magnesium are increased by the addition of charcoal to soils. The higher the ash content of the charcoal the greater the increase of these elements. As available nitrogen is apparently changed but slightly, if at all, the ratio of $N:P_2O_5:K_2O$ will be greatly affected, especially if the ash content of the charcoal is high.

16. Germination of white pine seed was reduced by the addition of charcoal to sand and loam. This reduction was at a linear rate with applications of 15, 30, and 45 percent of charcoal by volume. Fine charcoal reduced germination to a greater extent than did the coarse. Reduced germination is believed to be caused by an increase in the concentration of the soil solution due to the salts from the ash of the

charcoal, which decreases absorption of water by the seed.

17. The effect of addition of charcoal on growth of pine seedlings was not great. Only seedlings of those cultures containing fine hardwood charcoal (having a high ash content) differed in weight from the controls, the seedlings in the control cultures weighing more than those in the cultures containing fine hardwood charcoal. Seedlings in other cultures containing fine and coarse conifer charcoal (having a low ash content) and coarse hardwood charcoal did not differ in weight from the controls.

18. Height growth of seedlings was reduced with addition of charcoal, fine charcoal reducing the height more effectively than coarse. The reason for height growth being affected to a greater extent than weight is not understood. However, height growth and germination are affected in the same manner, and possibly reduction in height of shoots was caused by delayed germination and carried over into the second year.

19. Addition of charcoal to the soils had no effect on number of bifurcated mycorrhizae.

20. Damping-off of white pine seedlings was increased by addition of charcoal, but the infection was so light in all cultures that definite conclusions as to the effect of charcoal on damping-off could not be reached.

21. The number of colonies of heterotrophic, aerobic bacteria and actinomycetes; and fungi, as determined by the plate method, was not affected by the addition of charcoal.

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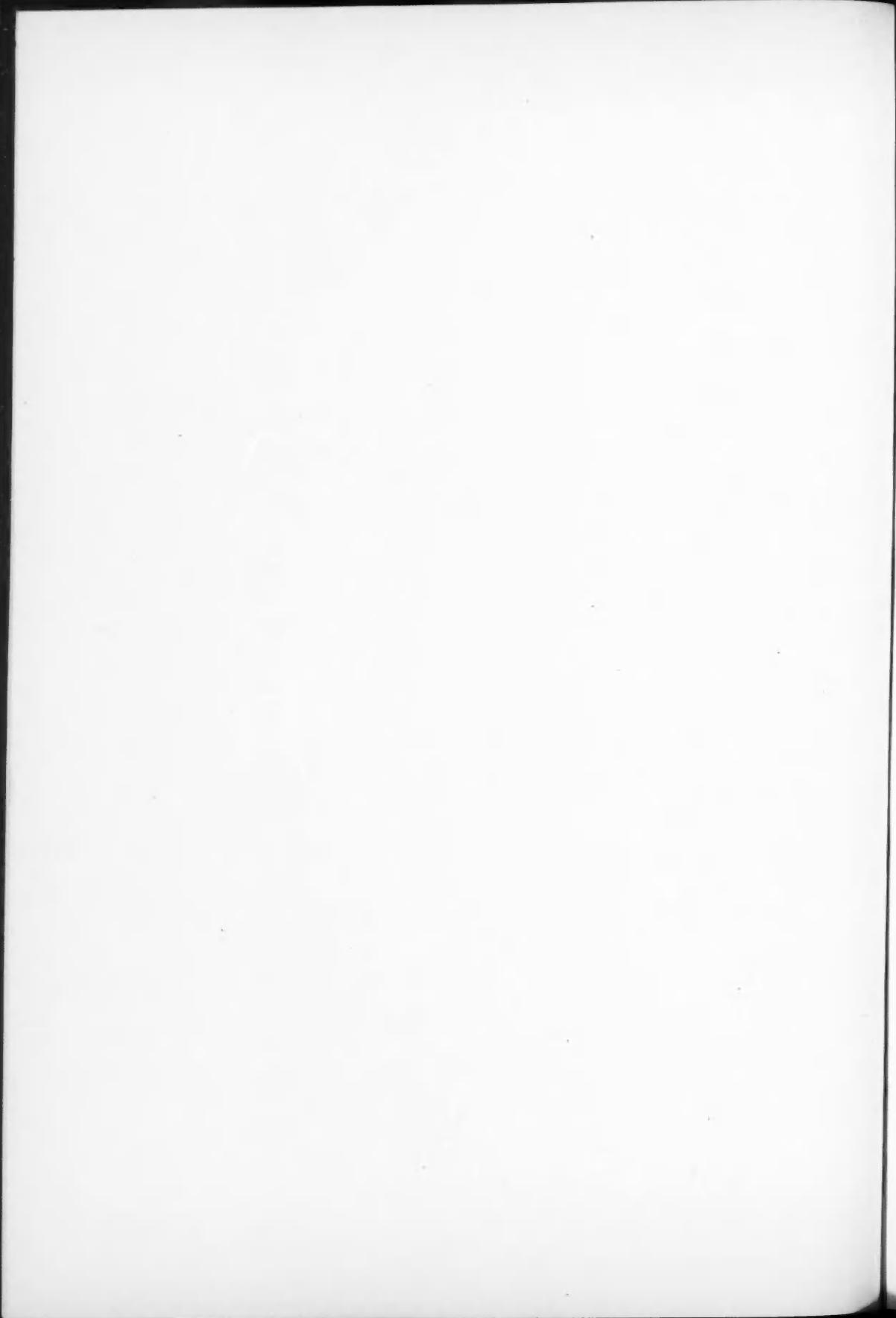
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ECOLOGY OF THE FOREST-TUNDRA ECOTONE ON THE
EAST COAST OF HUDSON BAY¹

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¹ Certain parts of this paper were taken from a thesis accepted by the Faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for a Ph.D. degree in March, 1942.

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ECOLOGY OF THE FOREST-TUNDRA ECOTONE ON THE EAST COAST OF HUDSON BAY

INTRODUCTION

The transition zone or ecotone between the Subarctic Forest and the Arctic Tundra Plant Formations in North America extends as a sinuous band across the northern part of the continent. The western extremity of the ecotone in Alaska has been studied thoroughly by Dr. Robert F. Griggs (1934). No intensive ecological studies have been made on the central and eastern portions of this vegetation zone.² In fact, it has been visited so rarely that our knowledge of even its exact geographic position is incomplete. This lack of knowledge is due to inaccessibility of the region and is not indicative of the potential significance of information on the ecology of this ecotone. The results of ecological studies in this region are valuable in themselves, and their usefulness is enhanced when they are directed toward the solution of problems of species distribution, the floristic and community history of the region, the process of plant succession, the more recent geologic history of the region, the character of the current climate, and as Griggs (1946) has so recently demonstrated, toward an understanding of the history of recent climatic variations.

In the summer of 1939, the writer had the privilege of studying a portion of this ecotone while assisting Dr. E. C. Abbe on the University of Minnesota Botanical Expedition to the Richmond Gulf Region. The primary objectives of the expedition were taxonomic and phytogeographic. Doctor Abbe, however, generously permitted me to carry on ecological investigations, releasing me from other duties whenever possible. My major concern was with tree growth-layer studies (tree-ring analysis) and their implications. Work upon the materials and data collected is not yet complete. The present paper deals with a particular problem—the forest-tundra ecotone, its nature and relation to environment, present and past. Growth-layer studies supply an important body of evidence in this connection and are used here as far as they are pertinent.

The writer wishes to record his sincere appreciation to Dr. E. C. Abbe and the societies that supported the expedition for the opportunity to carry out these investigations. Dr. W. S. Cooper generously assisted in the organization of data and preparation of the

² Due to the disruption of publication and of communications during and immediately following World War II, the paper, "Hustich, I, and Björn Pettersson 1945: Notes on vascular plants of the east coast of Newfoundland-Labrador. II. Memoranda Societatis pro Fauna et Flora Fennica 20: 24-46," in which Dr. Hustich's work on the eastern part of the ecotone is described did not reach the writer until after the present paper was in press.

thesis manuscript from which certain parts of the present paper were taken. Dr. Donald B. Lawrence was very helpful in selecting and securing field equipment, in conducting laboratory work, and in preparing the manuscript. Grateful acknowledgment is made for two research grants contributed by the Minnesota Academy of Science. Two months of very productive work were facilitated by Texas Technological College where the Biology Department supplied laboratory space, the Physics Department allowed free use of its lathe in polishing the sections, and Dr. R. A. Studhalter and Dr. Waldo S. Glock made many helpful suggestions. The wood-working shop of the Department of Mechanical Engineering at the University of Minnesota sanded the cross sections. Dr. F. C. Craighead, In Charge of Forest Insect Investigations, U.S.D.A., kindly arranged for loaning me the Bureau of Entomology's increment core measuring device. Dr. F. C. C. Lynch, Chief, Bureau of Geology and Topography, Canadian Department of Mines and Resources, generously supplied prints of Mr. A. P. Low's photographs. A research grant from the University of Colorado Graduate School defrayed certain expenses associated with preparation of the manuscript for publication.

GEOGRAPHY

Field work was carried out in the vicinity of Richmond Gulf, which is an extensive, irregular indentation just north of Latitude 56° North on the Hudson Bay coast of the Province of Quebec (Fig. 1). The region is of particular interest ecologically because it is located close to the north edge of the forest-tundra ecotone. The actual limit of tree growth, which reaches just north of Latitude 58° North in the interior of Quebec, swings southward as it approaches Hudson Bay and reaches the shore just north of Richmond Gulf (Fig. 1). Another feature which makes this region especially interesting is the fact that it lies only about 150 miles from one of the reputed centers of Labradorean glaciation (Low 1898) (Fig. 1). Its postglacial history has thus been short—perhaps not more than 10,000 years. For much of the surface the time available for colonization by plants has been still shorter, since, as Dr. George Stanley of the University of Michigan has shown, in immediate postglacial time sea level stood more than 800 feet higher than now.

Additional field studies were made near the mouth of Great Whale River which lies about 80 miles south of Richmond Gulf.

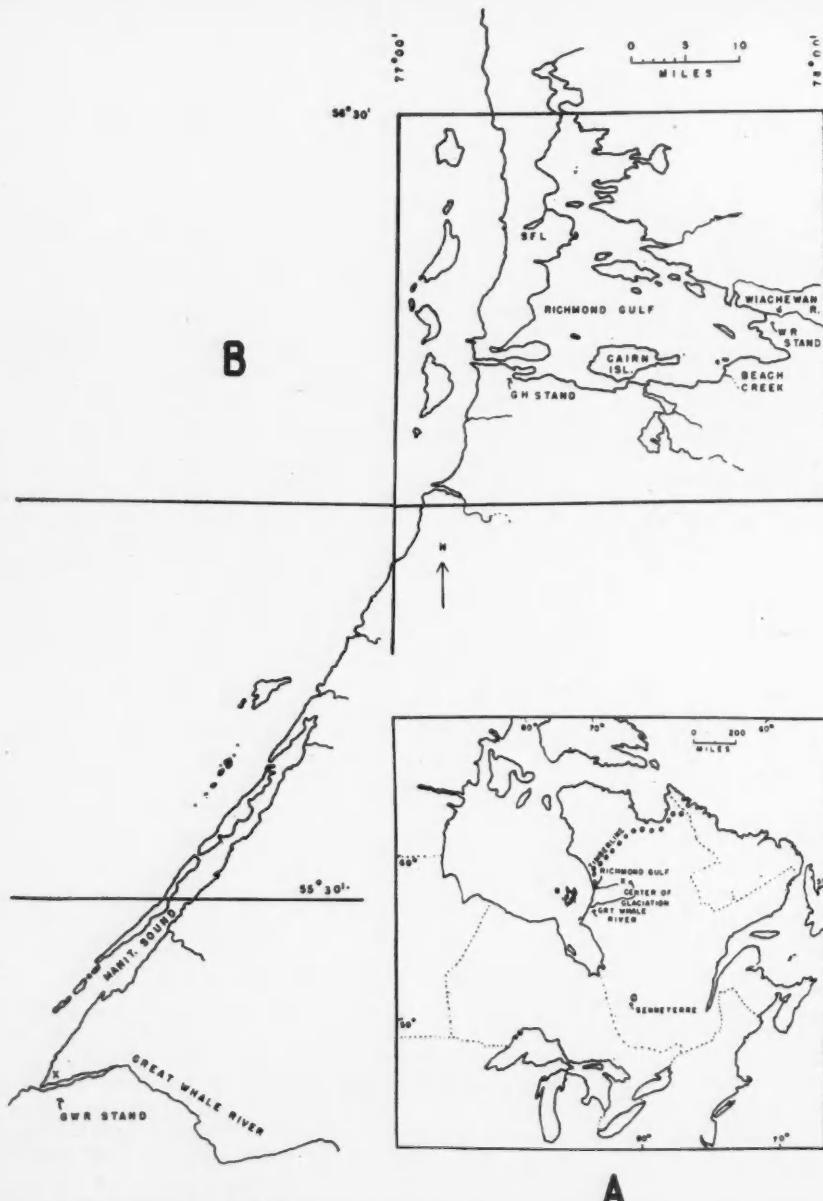


FIG. 1. A. Map of eastern Canada showing location of Richmond Gulf, Great Whale River, Center of glaciation, and northern timberline. B. Map of Richmond Gulf region and part of the east coast of Hudson Bay. Letters indicate location of stands of trees studied intensively.

GEOLOGY

In its lower reaches Great Whale River emerges from the low, rounded hills of the Laurentian Upland several miles from the shores of Hudson Bay and then meanders slowly through a broad channel cut down into its enormous "elevated delta" (see below). Along the coast of Hudson Bay north from Great

Whale River the monotonous landscape characteristic of southwestern Quebec is replaced by diversity of land forms and rock types, which results in equal diversity in soils and habitats. The scenery becomes progressively more spectacular northward until the climax of ruggedness is reached in the Richmond Gulf region. Farther north the topography is more sub-

dued. This local deviation from the regional topography is due largely to the presence of a long, west-facing, curving cuesta. In the south, this cuesta makes the line of islands which produces Manitouneuk Sound; in the north, it forms the separation between Richmond Gulf and Hudson Bay (Fig. 2). In the latter region the lip of the cuesta rises over 1,000 feet above the waters of Richmond Gulf and forms the highest land in the area. The cuesta is made up of many superimposed strata of Pre-Cambrian sedimentaries—both sandstones and limestones—covered by a continuous sheet of trap.

Some smaller cuestas, lower in altitude and roughly parallel to the main one, differing from it only in the presence of several basalt sills interbedded with the sedimentaries, extend this type of topography eastward along the south side of Richmond Gulf (Figs. 3 and 10). On the mainland south of Cairn Island, the topography changes abruptly to a more subdued type marked by rounded hills of the Laurentian gneiss and schist that make up the bulk of the Laurentian Upland. Similar hills occupy the area east and north of the gulf (Fig. 4).

Evidences of severe erosion by glacier ice is common in this region. Exposed rock surfaces are polished and often striated and grooved (Figs. 5 and 28) unless the character of the bedrock or the processes of subaerial weathering have obscured these

glacial phenomena. Glacial deposition was apparently restricted to a small amount of drift dropped in river valleys and probably a thin sheet of ground moraine laid over the uplands by the melting of the last ice sheet. Whatever deposits the ice left on the uplands have been largely stripped off by the action of running water and have been carried to the larger rivers.



FIG. 2. Aerial photograph looking southward along the cuesta which forms the narrow land separation between Hudson Bay and Richmond Gulf. (Royal Canadian Air Force Photo.)



FIG. 3. Looking southward from a hill on the south shore of Richmond Gulf showing topography and abundance of trees.



FIG. 4. Aerial photograph looking eastward into Wiachewan Bay and mouth of Wiachewan River. Hills in background show landscape characteristic of much of the Laurentian Upland. White area at head of Wiachewan Bay is an elevated delta. (Royal Canadian Air Force Photo.)

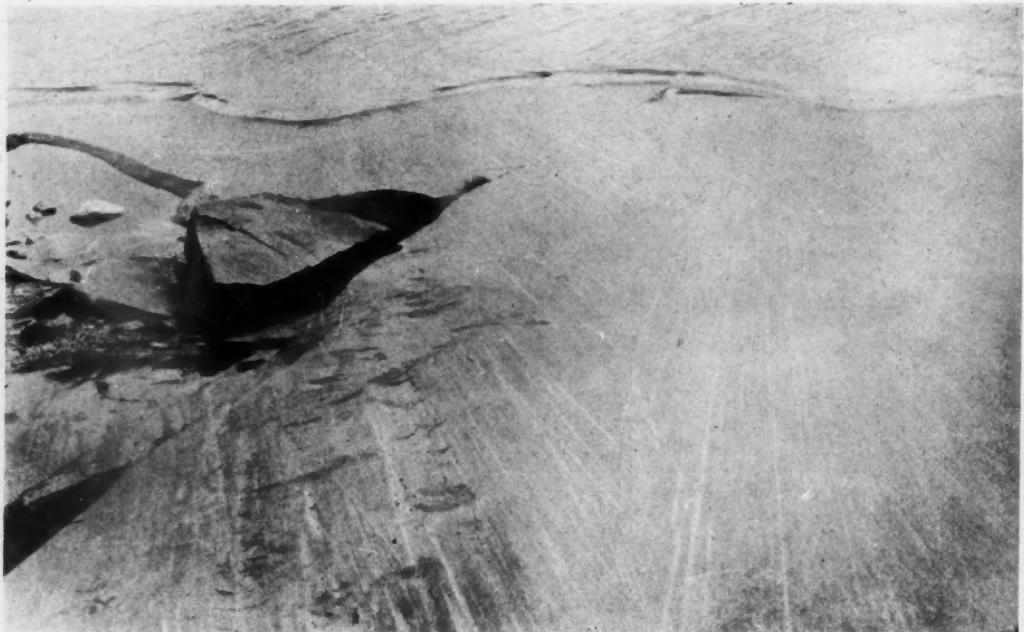


FIG. 5. Sandstone grooved, polished and striated by glacial action. East shore of Cairn Island.



FIG. 6. Looking northwestward across the mouth and elevated delta of the Wiaehewan River. The river now meanders along the north side (right side in the photograph) of the elevated delta.



FIG. 7. Profile through part of the elevated delta of Beach Creek. Southeast shore of Richmond Gulf.

An occasional glacial erratic boulder remains as a reminder of the previous existence of this drift sheet.

In post glacial time rivers have reworked the glacial drift deposited in their valleys and carried great quantities of it, along with that washed down from the uplands, to be redeposited in the form of flood-plains in the valleys and deltas at the river mouths. With relative lowering of the water level of Hudson Bay and therefore of Richmond Gulf also, the flood-plains have been trenched and thus converted into terraces and the deltas have emerged as dry land. These elevated terraces and deltas are very important in the present study. Excellent examples were seen at Great Whale River and at several points in the Richmond Gulf region (Figs. 4, 6, and 7).

Weather data for this region were fragmentary and unreliable until recent years. The period of careful and complete observations covers such a brief period that a thorough discussion of climate and weather is not justified. Data collected at Great Whale River Hudson's Bay Company Post by employees of the Company are complete enough to give a general indication of the temperature and precipitation (Table 1). No usable data are available for Richmond Gulf. However, since the Gulf is only 80 miles north of Great Whale River it is logical to assume that both temperature and precipitation will be slightly lower than the records for that station.

TABLE 1. Temperature and precipitation at Great Whale River, Province of Quebec. Average from record of 18 years.

	January	February	March	April	May	June	July	August	September	October	November	December	Yearly Average
Mean Temperature in degrees F.	-11	-9	1	19	32	43	49	51	45	35	20	2	23
Mean Precipitation in inches.	1.6	0.6	0.8	0.7	1.5	2.3	2.8	3.8	3.5	3.6	3.5	2.3	27

THE PROBLEM OF THE ECOTONE

In our flight northward from Senneterre, Province of Quebec, to Richmond Gulf (see Fig. 1) we passed in the space of a few hours over the whole breadth of the ecotone. South of James Bay, the earth was a patchwork of two colors, the monotonous green of the forest and the blue of innumerable lakes and rivers. As we approached the mouth of James Bay, a third color appeared, the grayish white of lichen tundra. First occurring in small openings in the forest, the tundra became increasingly widespread northward until it rivalled the forest in extent. Around Richmond Gulf tundra dominated; beyond, to the north, there were no trees and the gray tundra stretched unbroken.

The usual and quite natural hypothesis is that the transition from forest to tundra is due to a gradual transition in climate—that, with increasing climatic rigor, the forest is increasingly restricted to situations protected with respect to atmospheric factors. Ob-

servation from the air seemed to confirm this hypothesis—the forest patches appeared to be located in sheltered situations. Subsequent ground study revealed that appearance from the air was deceptive, that tree distribution had no apparent relation to shelter from unfavorable atmospheric factors. The obvious inference therefore was that climate is not a direct limiting factor for tree growth in this portion of the ecotone.

In order to test this inference, the trees were carefully studied in the field as to occurrence, size, growth-form, possible injury traceable to atmospheric factors, and general condition of vigor. An alternative hypothesis to account for the paucity of trees immediately suggested itself—limitation in occurrence and extent of areas where trees could obtain anchorage and adequate water supply, in other words, scarcity of soil in the broad sense prevents the development of forest over the entire region. This hypothesis was put to test in the field. Finally, materials were collected for growth-layer analysis, with the hope that such study would throw light on climatic conditions of the immediate past represented by the life-span of the trees.

FIELD OBSERVATIONS

TREE DISTRIBUTION IN RELATION TO SOILS

Around Richmond Gulf, slender strips of trees separated by tundra were seen from the air and it was assumed at the time that they were growing in protected valleys. The photographs in Figures 8 and 29 were taken on the ground in the area observed from the air. They demonstrate that the trees stand well above the surrounding area entirely unprotected by local obstructions. Actually, the pattern of tree distribution is produced by a series of narrow fractured belts which occur at regular intervals in the otherwise relatively unbroken bedrock surface. The intervening areas are polished rock with only a very thin covering of loose material. These fractured belts are lines of intermittent drainage along which the runoff from melting snow and occasional rains concentrates. Since water conditions are favorable and the crevices supply adequate anchorage, these belts support dense stands of relatively large and fast growing trees.

On Cairn Island and the mainland south of this island, folding, faulting, and differential erosion of the sedimentary and metamorphic rock strata have produced many series of narrow shelves. Most of these shelves slope inward from their outer rim to the base of the next shelf. Also, they are tilted at a low angle in the direction of the dip of the rock strata. Runoff naturally accumulates at the inner border of each shelf and produces there a relatively moist substratum. Therefore succession had proceeded rapidly and produced an organic soil deep enough to support trees. The outer portion of each shelf is usually too dry for trees (Figs. 9 and 10).

By far the most extensive forest stands and the best developed trees occur on the sandy alluvial de-



FIG. 8. The trees in the background form a narrow strip of forest along a zone of fracture in the bedrock. The relatively treeless lichen tundra area in the foreground occupies a strip of bedrock in which there are few cracks. Southeast side of Cairn Island.



FIG. 9. Scattered trees on narrow rock shelves on south slope of Cairn Island. The trees on these poor sites average about 10 feet tall.

positions of the river terraces and elevated deltas. For example, at Great Whale River a forest of thrifty, well-formed white spruce trees 40 to 50 feet tall stands on the exposed elevated delta at the mouth of the river (Fig. 11). These trees are absolutely unprotected from the prevailing winds from the west. At Richmond Gulf an equally exposed grove of well developed trees occurs on the southwest side of the long island west of Wiachewan Bay. Extensive forest areas occupy the alluvial terraces of the Wiachewan Valley (Fig. 12).

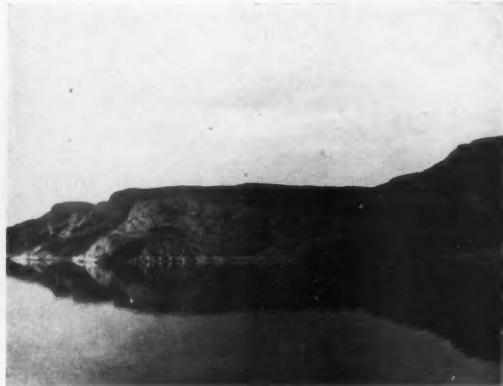


FIG. 10. Trees on broad shelf on mainland south of Cairn Island. Forest is migrating toward lip of shelf as soil develops. Also illustrates portions of several eustas on the south side of Richmond Gulf.

The absence of trees in other areas observed was found to be correlated with an absence of soil in almost every instance. The dearth of soil is related to the fact that this region lies close to a center of continental glaciation. The moving glacier ice removed practically all loose material from bedrock and



FIG. 11. Looking eastward across the surface of the elevated delta of Great Whale River. The delta slopes gently westward into Hudson Bay. Therefore the trees receive no protection from the prevailing westerly winds.



FIG. 12. Looking eastward up the Wiachewan valley from a hill near the mouth of the river. Well-forested areas are largely alluvial deposits. The uplands are hills of gneiss and schist characteristic of much of the Laurentian Upland.

left very little debris behind when it melted (Fig. 13). Most of what may have been left has been removed by stream wash, and also by wave action, which progressively affected much of the region during the relative lowering of sea level in post-glacial time. The period of subaerial history has been so short and the character of the climate is such that

very little weathering has occurred and therefore almost no residual soil has developed (Fig. 14). For the same reasons, the building up of a purely organic soil upon the rock surfaces has barely begun. Tree growth on these denuded areas is as yet impossible regardless of climate.



FIG. 13. Typical areas of bedrock from which all loose material was removed by the action of glacier ice and water. Southwest corner of Richmond Gulf.



FIG. 14. Typical area of bedrock exposed by action of glacier ice and water. The period of subaerial history has been so short that soil adequate for even herbs has developed only along the slight depression below the one foot high shelf. Cairn Island.



FIG. 15. Tree in foreground is typical for individuals growing on shallow soil. Mixed stand of larch and spruce in background has developed on the more favorable substratum along a zone of fracture in bedrock. The latter trees were about 30 feet tall. Interior of Cairn Island.

FORM OF TREES

There is considerable diversity in the form of trees in the eotone but it is related to variations in the type of soil and was never observed to be related to exposure alone. Trees growing from small rock crevices and in shallow peat-filled depressions are, as one would expect, dwarfed and straggling (Fig. 15). Those upon the deep, sandy soils are from 30 to 50 feet in height, straight-boled and, where the stand is open, with symmetrical crowns extending to the base of the tree (Fig. 16). Asymmetric individuals and "wind-cripples" are the rare exception and where they do occur are obviously related to very local conditions.

Field study thus indicated that extreme limitation of forest is due to paucity of areas in which soil conditions will permit tree growth. It revealed also that wherever there is good soil the quality of tree growth is high thus giving evidence that the atmospheric factors are also favorable for tree growth. In order to obtain more quantitative information, that is, to determine just how favorable atmospheric factors are, a study was made of the growth-layers of the tree.

GROWTH-RING ANALYSIS

FIELD METHODS

Trees reflect environmental influence through the quantitative and qualitative character of their growth-

rings. Consistently wide, clearly annual rings indicate favorable growing conditions, whereas rings that are consistently narrow, erratic in relative width, incomplete, or non-annual indicate unfavorable conditions.

At the present stage in growth-ring research it is not possible to relate ring characters to any one environmental factor. It is, however, entirely possible to group these factors into three categories: atmospheric, edaphic, and biotic. It is possible, further, to select materials in such a way as to minimize the effects of edaphic and biotic factors, so that the character of the growth-rings may be traced to the atmospheric category as principal cause. Trees growing in favorable soils and showing no sign of injury fulfill this requirement.

The large size and thrifty appearance of the trees on elevated deltas indicated that, among the various types of substrata present in the region, the sandy alluvial soils of this land-form were the most suitable for tree growth. It was concluded therefore, that in the growth-rings of these trees effects of atmospheric factors would be emphasized and influence of edaphic factors minimized. The soil of these elevated deltas was observed to be essentially uniform in character within a stand of trees and from one area to another,



FIG. 16. Spruce forest on elevated delta of Great Whale River.

and therefore unlikely to be a disturbing influence in comparing tree growth in the different localities.

The effects of certain other factors which might produce abnormalities in tree growth not related to climate were guarded against as far as possible. Individuals were selected which grew in the interior of the stands, since these presumably would not be subjected to asymmetric competition or unequal exposure to wind. An attempt was made to select trees similar in crown form, since it has been suggested that irregularities in crown form affect the circumferential development of xylem in the trunk (MacDougal 1936). There remains the possibility of genetic differences between individuals, expressing themselves in differing physiological behavior. The only precautions here possible were to concentrate on a single species and to select individuals which were externally similar.

The conifers of the region are white spruce (*Picea glauca* (Moench) Voss), black spruce (*P. mariana* (Mill.) B.S.P.) and larch (*Larix laricina* (Du Roi) Koch). White spruce is the most abundant and best developed species on the elevated deltas, and therefore was selected as the major species for growth-ring analysis. The other two species were also studied to some extent.

A preliminary study of collections from larch reveals that extreme fluctuations in annual growth occur more frequently in this species than in spruce, and suggests that it is better suited for certain types of growth-ring studies. However, since larch has more peculiar and possibly non-annual rings than spruce, it is desirable to investigate more thoroughly before reporting on that species.

Materials collected for studying the radial growth of a tree generally consist of several increment cores taken at breast height or a cross section taken at the base of the tree. The former method is obviously dependable only when radial growth is uniform throughout the tree. It has been suggested that trees growing at progressively higher altitudes or latitudes become more and more erratic in radial growth, producing more than one layer a year, or incomplete cylinders of xylem, or some other type of abnormality. Therefore, it was anticipated that in the Richmond Gulf region there would be considerable variation in growth within a single tree. Recognition and understanding of these confusing anomalies is practically impossible from increment cores. It is a mere assumption, too, that the basal portion of the tree is most sensitive to the influence of environmental factors and most accurate in recording their effects. It is more reasonable to assume that, as Glock has pointed out (1937), complete knowledge of the relations between tree growth and environment can be obtained only by complete dissection of the bole. This is obviously impractical, but a series of sections at different levels taken from the trunk of a single tree provides a method that is workable. It would be desirable, naturally, to sample in this way a number of trees in each stand, but lack of time and limitations of transportation made this impossible. In the light of experience of other workers it was considered that a series of cross sections from one tree and four cores from each of about ten others constituted a sample sufficiently large to give a reliable record of average growth for each stand.

The procedure followed in making collections from each suitable stand was as follows: The largest symmetrical tree was selected for dissection. Cross sections 2 to 3 inches thick were taken at five-foot intervals beginning one foot above the soil (basal flare was not evident in this region). The north side of each section was marked and the collection number was printed on the top of the section. A portion of the crown was collected so that field identification of the species could be checked in the laboratory.

A Swedish increment borer was used in obtaining cores from additional trees. Although the previously described characteristics were used in selecting individuals from which cores were taken, slight irregularities such as bushy tops in older trees could not be avoided. The four borings were made in line with the cardinal points of the compass. The collection number of the tree and the compass direction of the core were marked on each with permanent ink. Cores were placed in aluminum tubes,³ in which small quantities of naphthalene flakes were placed to prevent growth of fungi. Each night the tubes filled during the day were transferred to the "storage case" which was always left in camp.

DESCRIPTION OF STANDS

Two stands of trees were studied intensively. One of these was on the almost level elevated delta at the mouth of Great Whale River (see Fig. 11). The forest here was magnificent for an area only 80 miles from the northern limit of trees. The trees averaged over 35 feet tall; most of them were almost perfectly symmetrical, and were spaced far enough apart to permit survival of branches all the way to the ground. The community had the general aspect of parkland. A continuous mat composed of several species of Cladonia lichens 6 to 12 inches tall covered the ground between the trees (Fig. 17). White spruce comprised the bulk of the forest; black spruce was absent over much of the area, but did occur near the contact of the alluvial deposits with the hills to the east.

The other stand grew 80 miles north of Great

³ Having in mind the difficult conditions of travel to and within the Richmond Gulf region, Dr. Donald B. Lawrence of the University of Minnesota and the writer devised a method of carrying cores which proved to be very satisfactory. The carrying tubes were made of Alcoa (half-hard) aluminum tubing with 0.035 inch thick walls and outside diameter of 3/16 inches. The tubes were cut into 16-inch lengths. The entire set of tubes was numbered consecutively with metal dies, each one at both ends. Small cork stoppers served to close both ends. Two cases made from tough, cardboard mailing tubes were used for carrying the tubes. The smaller was 46 cm. long and 5 cm. in diameter, accommodating 28 tubes. This was obtained in finished form with metal ends and a slide-on top which was held in place with a rubber band. A larger case was constructed by placing round wooden blocks in the ends of a mailing tube 50 cm. long and 8 cm. in diameter. One block was nailed in place, the other fitted with a bolt and wing-nut cap. This case accommodated about 70 tubes. Both cases were covered inside and out, with a coat of valspar varnish. The larger, complete with tubes, weighed 4 1/2 pounds. The smaller weighed one pound ten ounces. The latter was used as a "field case"; the larger was kept at base camp and served as a storage case for both filled and empty tubes.

An unexpected advantage of these aluminum tubes is that they are practically air-tight. The cores retain their moisture and do not shrink, and shaving, which is necessary for study, is easier and produces a smoother surface than is possible with dry cores. A year after collection it was possible to make free-hand sections without previous soaking for the purpose of studying the cambium and outermost xylem cells.



FIG. 17. White spruce and ground cover of Cladonia lichens in the Great Whale River Stand. Some of the trees were over 40 feet tall; the average was about 30 feet.

Whale River just south of Gulf Hazard, the strait connecting Richmond Gulf with Hudson Bay (see Figs. 18 and 19). The elevated delta here slopes rather steeply from the base of the high cliffs down to the beach. The white spruces averaged over 40 feet in height but, due to the denser character of the stand, some were not as symmetrical as those at Great Whale River. Although the trees at the lower elevations were generally more symmetrical than those higher up, their roots lay so near the water table that their use in this study was clearly ruled out. The trees from which collections were made were 50 feet or more above the level of the beach.

A forest of black spruce 20 miles east of Gulf Hazard growing on the elevated delta of the Wiacheewan River in the southeast corner of Richmond Gulf was also studied. Cross sections were taken from one large tree (No. WR 129, Fig. 20) that possessed some of the irregularities noted in the other stands. Consideration of the growth pattern of this tree therefore strengthens the conclusions drawn from the slightly asymmetric trees in the larger stands described above.



FIG. 18. Aerial photograph showing area of Gulf Hazard Stand which is indicated by an arrow.



FIG. 19. The Gulf Hazard Stand of white spruce. Trees averaged over 40 feet tall with some over 50 feet tall.



FIG. 20. Tree number WR 129, a 37 feet tall black spruce with irregular longitudinal distribution of branches. Elevated delta of the Wiachewan River.

LABORATORY METHODS

CROSS SECTIONS

Preparation of the cross sections was begun in the wood-working shop of the Department of Mechanical Engineering at the University of Minnesota. After major irregularities had been removed with a circular saw, the sections were sanded under a large "band-sander." The grade of sand paper used was too coarse to produce a uniformly well polished surface, but the distinctness with which the growth layers stood out on well sanded portions and the speed of operation suggests excellent potentialities for this method.

The sections were later taken to Texas Technological College, where Dr. Waldo S. Glock very kindly demonstrated an effective method of polishing them, which is similar to the one independently attempted by the writer. Using Dr. Glock's technique, the sections were treated as follows:

A section was clamped to the revolving head of a lathe. If it had an irregular surface, initial leveling was accomplished with a sharp lathe knife. While using the knife, it was necessary to have the section revolving at high speed and only a very minute thickness could be cut at one time. The depth of each cut and the rate at which the knife moved across the

face of the section were gradually decreased until only powdered wood was being removed.

After smoothing with the knife had been completed, sand paper mounted over a block of wood was moved back and forth across the face of the revolving section, successively finer-grained papers being used. During the final polishing, in which felt was used in place of sandpaper, it was necessary to keep careful watch for the point where there was maximum differentiation between the "light wood" (spring wood) and "dense wood" (summer wood) and therefore maximum distinctness of growth layers.

The surface produced by this method is very well suited for growth-layer studies (Fig. 26). Since individual cells stand out very clearly when viewed with a lens, it is easy to locate the absolute limits of a layer.

After polishing was complete, six radii approximately equi-distant and apparently free from abnormalities were selected for measurement. Along each selected radius every 10th, 50th, and 100th layer was marked with one, two, and three needle holes respectively.

Measurements were made with an increment core measuring device which is the property of the Bureau of Entomology, U. S. Department of Agriculture. The instrument is essentially a binocular microscope with the stage attached to a threaded bolt with knobs on the ends for moving the stage. A dial-recorder connected with the stage records the horizontal distance which it moves. This connection is fitted with a release attachment which when tripped permits the indicator needle to return to the zero position, thus allowing direct reading of the measurement of each ring and increasing the speed of operation. The recorder is graduated in 0.01 mm. divisions. Numbers appear at 0.1 mm. intervals and one complete revolution of the needle represents 1 mm. Thus measurements can be read accurately to a fineness of 0.01 mm.

Since an individual growth-ring has a somewhat undulating rather than a smooth-walled surface, following a straight line from bark to pith results in oblique measurement of some layers. Therefore, all measurements were made parallel to the rays so that they would represent the same dimension of each layer.

The accuracy of the instrument was tested by duplicating the measurements of over 800 rings. Comparison of the two sets revealed an average variation between the duplicated measurements of only 0.04 mm. and a maximum variation of less than 0.1 mm. Since no marked line was followed between the needle-holes on the section, these variations may be due to failure to duplicate the original line of measurement as well as to possible errors in the instrument.

Since each growth-ring was measured 36, 30, 24, 18, 12, or 6 times, depending on its position in the trunk, it was possible to check the measurements by comparing graphs of the measurements. For ex-

ample, the measurements of each growth-ring on all 6 radii of one section were added together and an average width for each growth-ring was obtained. These data were then graphed. Thereafter, the points for the graph of a single radius were plotted but before connecting lines were drawn the points were compared with the average by superimposing the two. When the width of a ring on one radius was conspicuously different from the average for that layer a check was made against the measurement data sheet, then against the section. When the discrepancy on the graph was not obvious on the section, duplicate measurements were made. Thus, if a given ring was relatively thick on the average, and thin on a single radius, a complete checking was made.

The average width of the rings on each cross section was obtained by averaging the measurements from the 6 different radii. The average width of the rings in a tree was obtained by averaging the data from all sections from the tree.

INCREMENT CORES

Increment cores were prepared for study by shaving one side with a sharp razor blade; an attempt was made to provide a surface which cut the tracheids exactly in transverse section, because in that plane the growth rings are most distinct. The rings were counted from the outside inward, and, as in the cross sections, each tenth ring was marked with a needle hole.

Since the cores were intended to supply data revealing only the more conspicuous fluctuations in growth, it was not necessary to measure the rings. The more rapid "reading" technique developed by Douglass and fully described by Glock (1937) was used. The cores were read by means of a low-power binocular dissecting microscope. The critical narrow rings (narrow in relation to neighboring rings) and other peculiarities were recorded on a data sheet, producing a so-called "number plot." The data from the four number plots of a single tree were averaged and recorded on what may be called a chart of critical rings on which the height of a line is inversely proportional to the relative width of the ring it represents. Finally, these were combined to give a diagram of critical rings for each stand.

Laboratory study of the cross sections revealed that in a few cases the innermost rings at one level were conspicuously different from the width of those rings at lower levels in the trees; therefore the inner 10 rings were not considered.

Actual measurement would doubtless reveal numerous additional rings which are consistently below average width but in which the differences are too slight to be detected with certainty by the technique used. As a matter of fact while studying the cores, the writer was conscious of numerous slightly narrower rings which seemed to cross-date as accurately as the conspicuously narrow ones but which were not "critical" enough to take on unquestionable significance.

METHODS OF PRESENTING DATA

CROSS SECTIONS

The characteristics of the growth-layers which bear upon the present problem are given in graphic form. Figures 21 and 22 present the data derived from cross sections of the two dissected white spruces, GWR165 and GH152, respectively, upon which main reliance has been placed. Figure 23 gives similar data for black spruce, WR129.

Since it is difficult to compare slight differences when the graphs are placed one above the other on a single sheet, a special technique was developed for reproducing the graphs in combination. The measurements from each radius for a given section were plotted on separate sheets of tracing paper tacked over 2-mm. graph paper and the plotted points were connected to make a continuous line. Any desired combination of graphs could then be compared by superimposition and viewing by transmitted light.

For illustrative purposes, the graphs to be compared were superimposed, placed between glass plates, and photographed by transmitted light.

Each set of 5 or 6 superimposed graphs (A through E or F) in Figures 21, 22, and 23 thus contains the measurements made on a single cross section; each constituent graph represents a single radius. Each graph of the set at the bottom (AVS) represents the average width (from 6 measurements) of the growth rings on a single cross section.

INCREMENT CORES

For organizing the data from increment cores a technique more rapid than measurement was employed, since in this case the absolute width of the rings is not needed. The distinctively narrow rings, properly spaced so as to indicate their positions in the time sequence, were recorded on 2-mm. graph paper. In Figure 24, based on 8 trees from the Great Whale River stand, each vertical line represents the occurrence of a growth-ring visibly narrower than its neighboring rings and by its length the approximate degree of narrowness. The intervening blanks indicate periods during which layers of approximately average and uniform width were formed. (This technique is taken from Douglass's and Glock's method in dendrochronology building as described by Glock in 1937.)

The data from the individual trees in the Gulf Hazard stand are very similar to those from Great Whale River. Master charts from the two stands representing departure from average layer width are constructed by averaging the lengths of the lines given in Figure 25.

RESULTS: CHARACTERISTICS OF GROWTH-RINGS

ANNUAL CHARACTER

A necessary prerequisite to this type of growth-ring study is to know whether or not each individual layer was actually built up in a single growing season. The usual assumption is that, with rare and

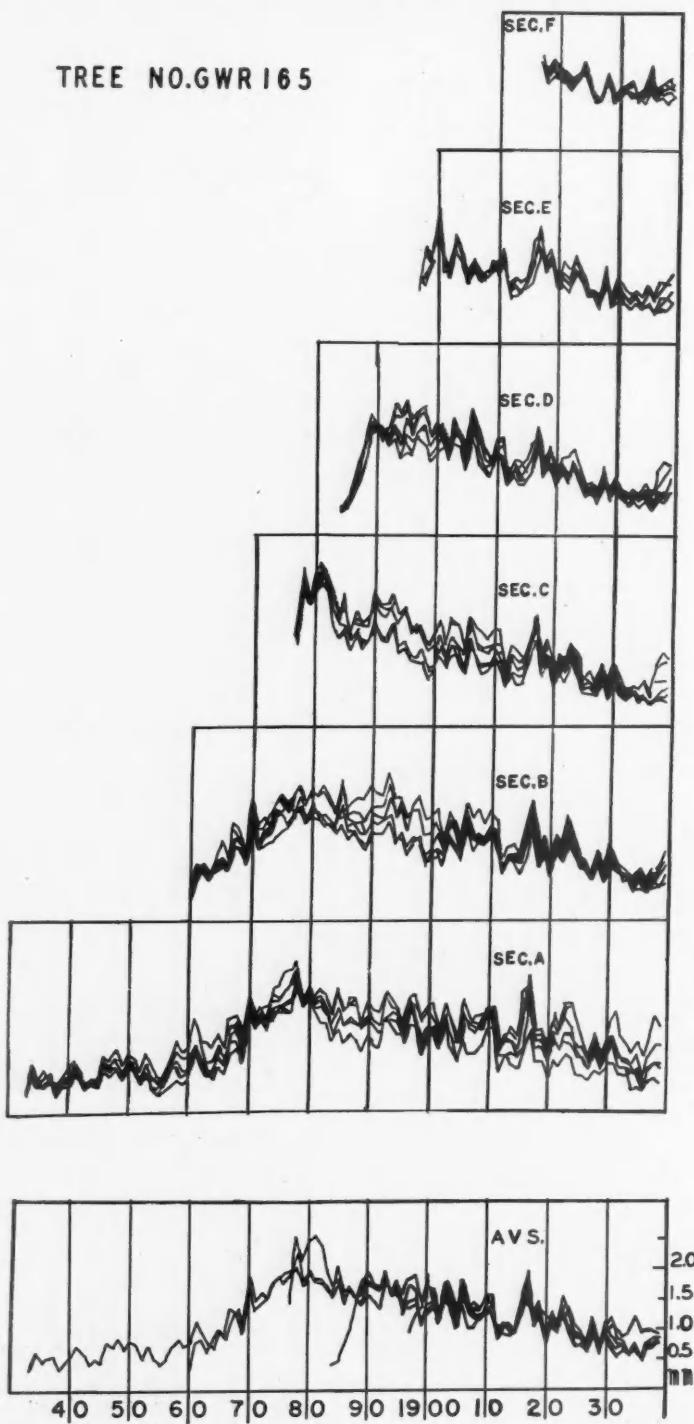


FIG. 21. Graphs of measurement of growth rings of tree number GWR 165. See text for explanation.

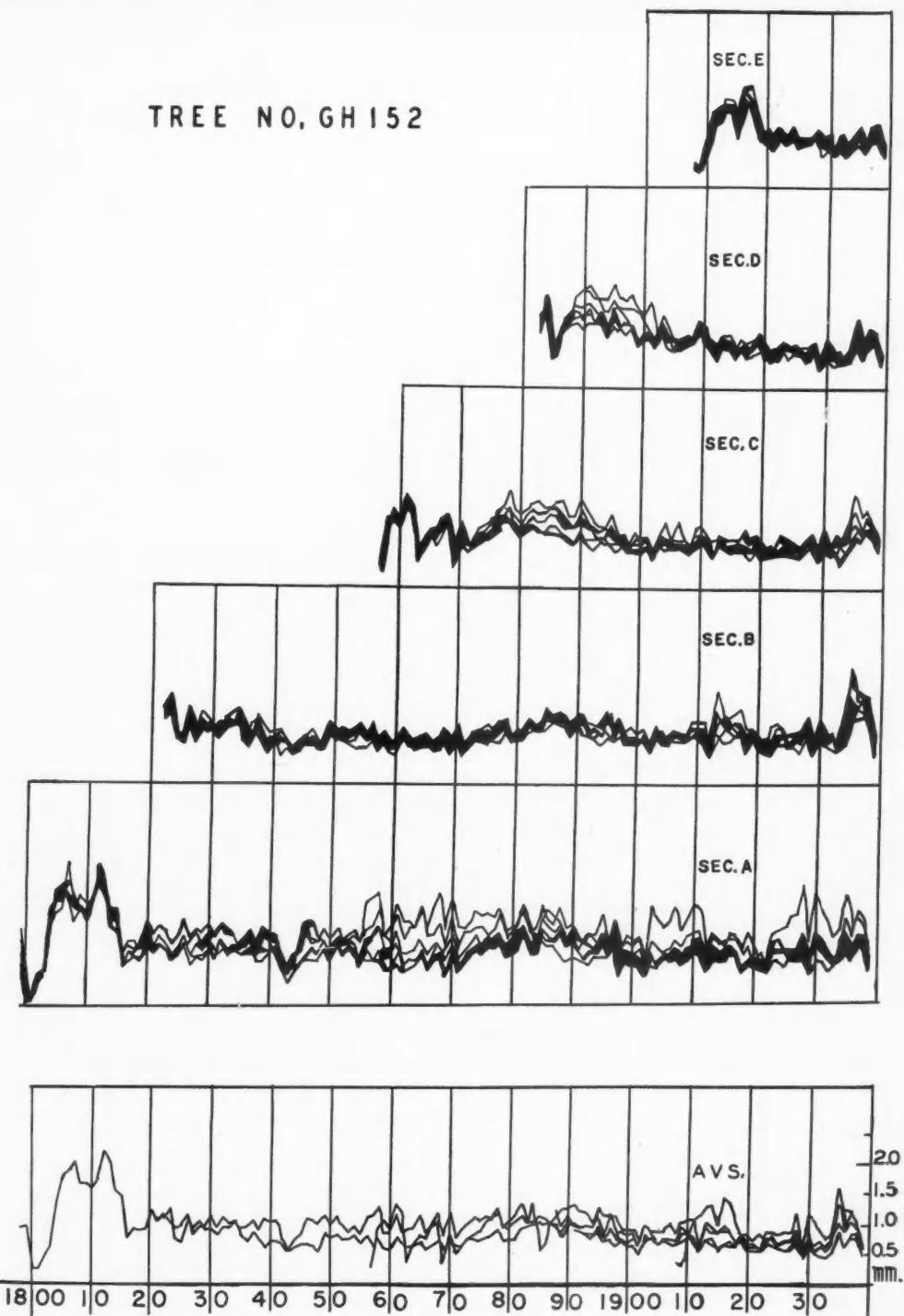


FIG. 22. Graphs of measurements of growth rings of tree number GH 152. See text for explanation.

TREE NO. WR 129

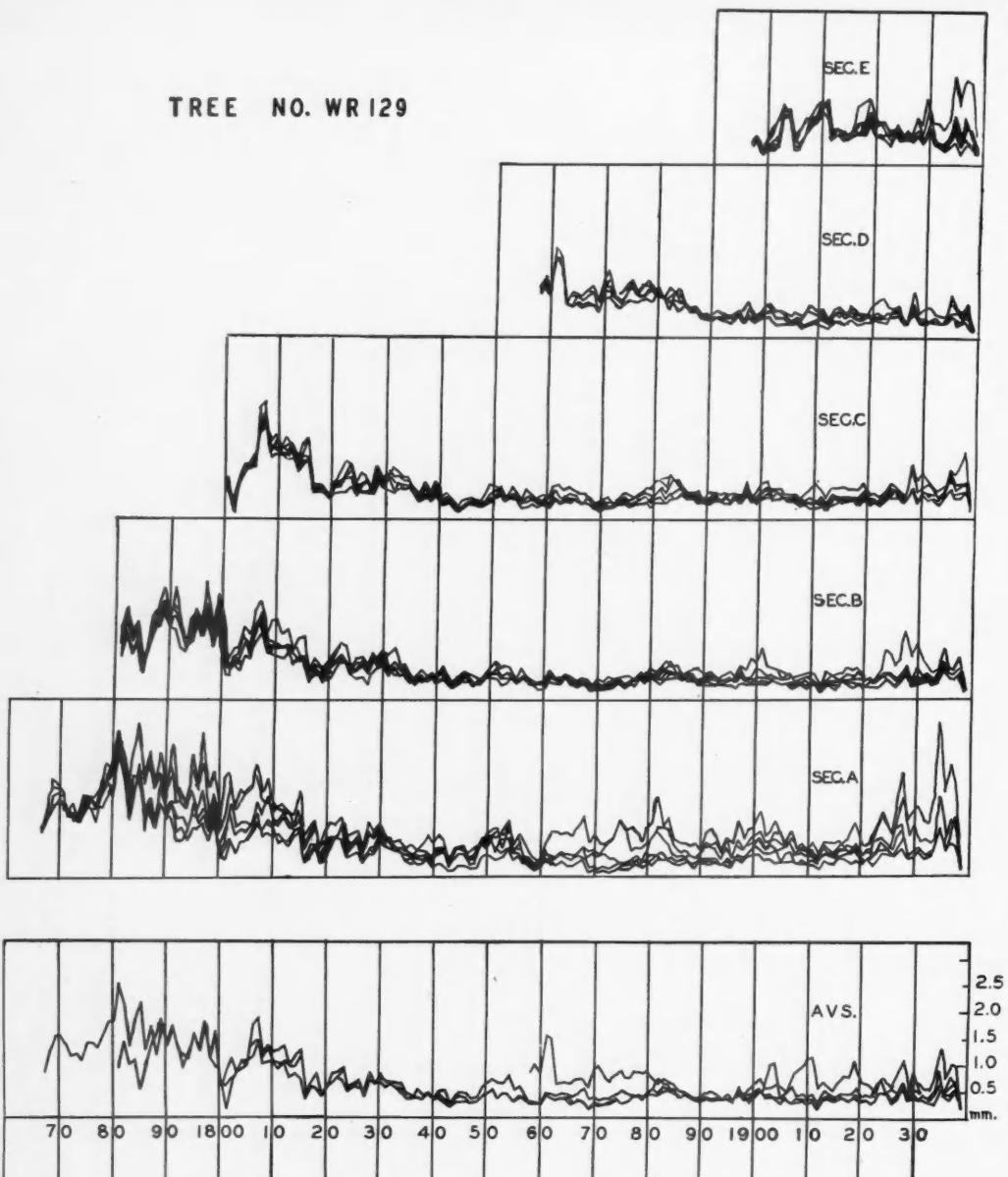


FIG. 23. Graphs of measurements of growth rings of tree number WR 129. See text for explanation.

easily identified exceptions, growth-rings are annual. Glock believes, however, that one should not make this assumption without considerable supporting evidence. He states in particular that "the possible presence of multiple growth-layers in one annual increment should be taken into consideration by those who take their trees from border areas" (1941). The Richmond Gulf region is assuredly a "border area," and therefore caution must be used at this point. The

confusion which would arise from attributing two or more very narrow layers formed in a single season to successive seasons is perfectly obvious.

The writer is confident that in the spruces used in this study the growth-rings are predominantly, if not entirely, annual—that if any extra layers are present they are so rare as to be of no consequence for this study. This belief is founded on a detailed study of the course of growth in 1939 and is further supported

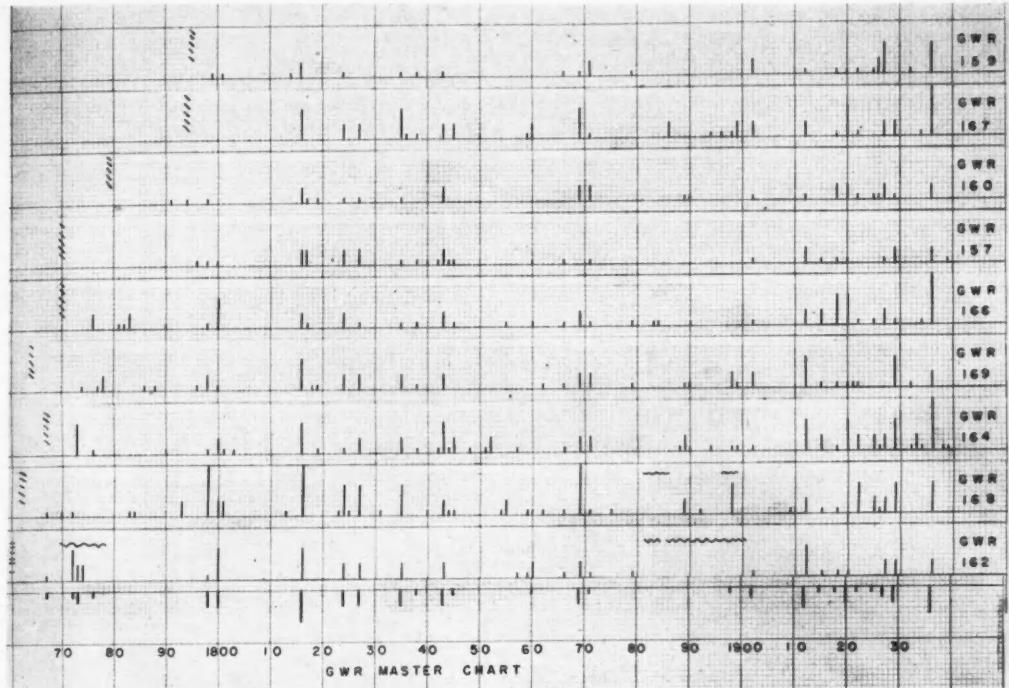


FIG. 24. Charts of critical rings for 9 white spruce trees in the Great Whale River Stand and a master chart for the entire stand. The height of each heavy line is inversely proportional to the relative width of the growth ring for that year.

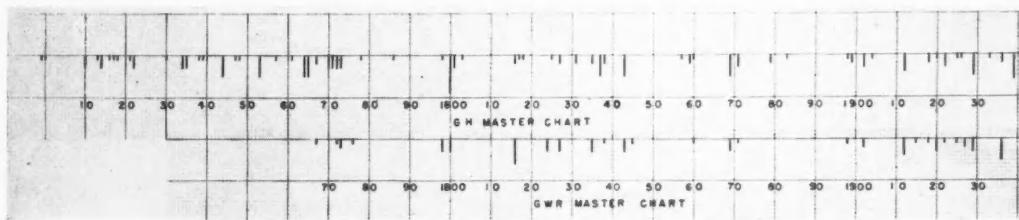


FIG. 25. Master charts of critical rings for the Great Whale River and the Gulf Hazard stands.

by deductive reasoning. During the summer of 1939 collections were made every week from the first week in July through the month of August. A single complete ring was formed during that period and it is very improbable that another layer could have been produced in the short time remaining before the freeze-up. The brevity of the aggregate time in which temperature is high enough for the cambium to function suggests that the production of several layers in one season is almost impossible. Brevity is due first to the shortness of the growing season, which probably averages less than two months, and second, to the low average daily temperature (around 50° F.) during that season, which still further limits the time during which the cambium can function.

Certain rings having extremely narrow dense wood

(Fig. 26) (Apparently comparable to the "light late-wood rings" of Giddings (1941).) might be suspected of being "false annual layers." This is not possible, however, because this type of dense wood may occur in two successive and relatively wide rings. If we assume that these are false annual rings, we are assuming that the narrow dense wood was formed in the middle of rather than at the end of the growing season. This would further require that with such layers there is associated another ring having the normal dense wood formed at the end of the growing season. Therefore if the two adjacent layers with narrow dense wood were both false annual rings, it would mean that three complete layers, two with narrow dense wood and another with the ordinary type, were formed in a single year. The product would

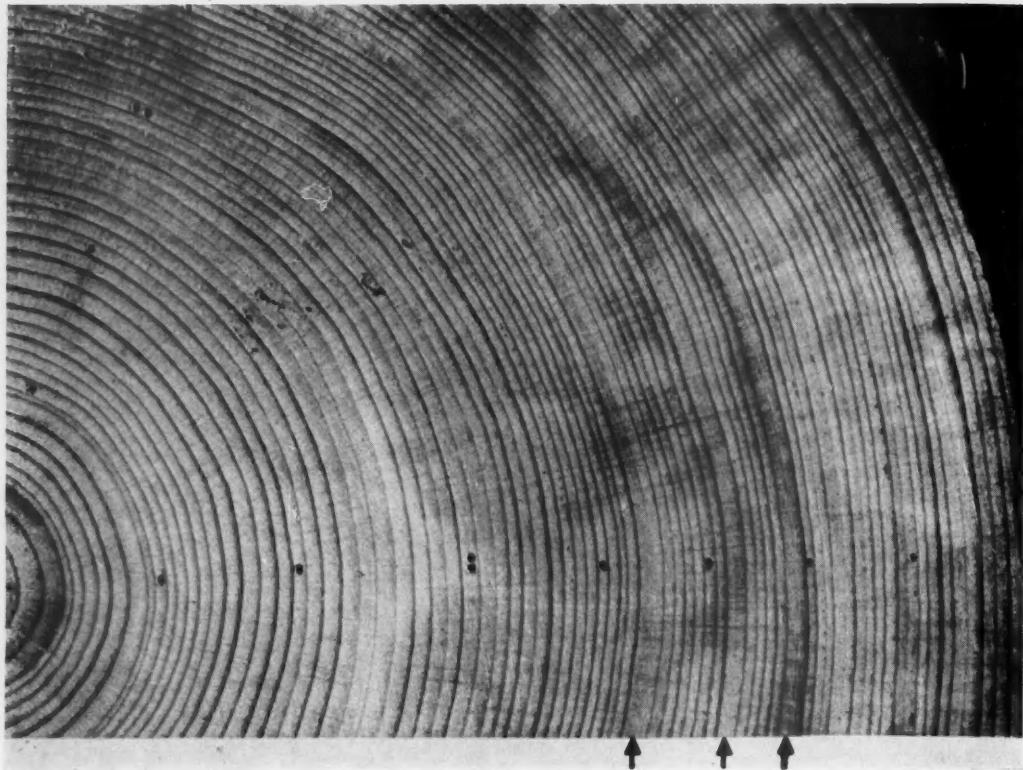


FIG. 26. Part of a polished cross section of a tree illustrating growth rings having extremely narrow dense wood (indicated by arrows).

be a layer of xylem about twice as thick as the widest single ring observed. It is highly improbable that a single growing season interrupted twice by abnormally adverse conditions (indicated by the layers of dense wood) would permit the production of so much xylem. A more logical explanation of these layers with narrow dense wood is that conditions permitting radial growth came to an abnormally abrupt ending rather than tapering off gradually and permitting the development of the usual amount of dense wood.

AVERAGE WIDTH

Average width of growth rings is roughly indicative of average quality of growing conditions. Since the trees here studied grew upon the comparatively favorable soils of the elevated deltas, it may be assumed that any limiting effect of edaphic factors was very slight and that, therefore, the average width of their growth-rings indicates the general suitability of the atmospheric factors for their growth. Table 2 presents data on the average width of growth-layers of trees in three different stands on elevated deltas.

Layer width is decidedly small in comparison with what one would find in the heart of the conifer forest region; but yet, the average for all, approximately

1 to 2 mm., is by no means the almost microscopic figure that we associate with trees growing under conditions approaching the limit for existence.

NARROW LAYERS

Frequency and severity of unfavorable years are more significant in evaluating climatic suitability for forest growth than are average conditions; such seasons are recorded in the bole of a tree usually through rings narrower than the average or by incomplete layers. If narrow layers "cross-date" (occur in the same year) from tree to tree over a wide area, it may be assumed to be caused by adverse weather conditions. The only other factor that varies uniformly over a wide area from year to year is attack by parasites. No evidence of injury by parasites was found in the Richmond Gulf region.

In order to cover a sufficient number of trees to permit an adequate attempt at cross-dating it was necessary to depend in the main upon increment cores. Since the width of a growth-ring may vary somewhat between different sides of a tree, it was necessary to determine whether or not measurements on four cores cover the range of variation completely enough to give a reliable picture of the relative radial growth of a tree. This was done by resorting to the more com-

plete and quantitative data provided by the several sections of the three dissected trees.

Comparison of the relative width (relative to its two neighboring layers) of a growth-ring on different radii of a cross section gives a character called "eircuit uniformity" by Glock (1937). Tree GWR165 has essentially perfect circuit uniformity on all six cross sections⁴ (Fig. 21). "Vertical uniformity," the relative thickness of a layer at different levels in the tree, is also essentially perfect. This is demonstrated in Figure 21 AVS which shows the averages of all sections superimposed. With the exception of the central few layers at each level, a growth-ring has the same relative width at all levels. The central rings at any level above the base may fail to vary in accordance with the same layer at lower levels. For example, the 1922 layer is relatively wide (wider than the 1921 and 1923 rings) at level F, where it is only three layers from the center, while on all lower levels, where it is farther from the center, it is relatively narrow. The perfection of circuit and vertical uniformity is emphasized by the fact that out of 2,340 measurements of 107 different layers, only one layer on two radii of one section fails to conform in having essentially the same relative width in all parts of the tree. For tree GH152 the comparative graphs in Figure 22 reveal that both "eircuit" and vertical uniformity are very good. In a single case it was found that an abnormality had upset the normal sequence of relative thickness of layers. On radius three of section B, the 1922 layer is as wide as the 1921 layer, while on all other radii it is relatively narrow. Inspection of the cross section showed that this discrepancy is due to a local "surge" in the 1922 ring on radius three.

Although GH152 had a symmetrical crown, some of the older trees in the same stand from which cores were taken had slightly irregular longitudinal distribution of branches. It was suspected that irregular growth rings might be associated with this feature. In order to determine whether such a connection existed, an analysis was made of WR129, a black spruce which had the most irregular longitudinal distribution of branches of any tree encountered. Figure 23 shows that in spite of longitudinal irregularities in its crown (Fig. 20) and considerable quantitative variation in growth in different parts of the tree (A and F of Fig. 23) both eircuit and vertical uniformity are essentially as perfect as in the other two dissected trees. It appears, then, that the trees at Gulf Hazard with slightly irregular crowns may safely be used in this study.

The above conclusions drawn from the three dissected trees indicate that the amount of growth in one year relative to that of the preceding and following years is constant in all parts of the bole except in the innermost few rings at a given level. Study of a single core taken at any point, therefore, gives an accurate representation of the relative width

of the growth-layers in trees of this region and an average of data obtained from cores along four radii is more than adequate. It should be emphasized that these statements apply to relative growth only and do not hold true for the absolute quantity of growth.

The reliability of cores having been established, it is next in order to consider the results obtained from these samples. Examination of Figure 24 giving data from 8 trees at Great Whale River, each tree represented by four cores, shows that the critical narrow rings correspond in a regular pattern from tree to tree—in other words, that they cross-date. The diagrams for the other 6 trees from this stand are not given here because they are relatively young. It is significant, however, that the narrow layers in these younger trees cross-date with those illustrated in Figure 24. The cause of production of narrow rings is thus operative not in individual trees merely but throughout the stand. Comparison of the master charts of Great Whale River and Gulf Hazard (Fig. 25) demonstrates satisfactory cross-dating of narrow layers between these two stands, which are 80 miles apart. Cross-dating is equally close between the sections of the dissected trees from the GWR and GH stands (see Figs. 21 and 22). The cause of production of narrow rings is therefore regional in operation. The only factors which fluctuate regionally in this area are atmospheric. The reasonable conclusion is that variations in relative width of growth-rings are due to fluctuating climate, and that the critical narrow layers were formed in particularly unfavorable seasons.

The above conclusions are based upon trees growing in one type of soil, and, with a single exception (WR129), upon one species. Preliminary study of collections from spruce growing on clay and in rock crevices, and from larch on peat and sand reveals that the conspicuously narrow layers in these trees cross-date with those in the trees growing on elevated deltas. Fluctuations in growth that cross-date from one soil type to another and from species to species are added confirmation of the above conclusions.

INCOMPLETE LAYERS

Examination of the three dissected trees revealed not a single instance of a layer that was circumferentially incomplete. Vertical continuity is equally certain, since the layers could be accurately cross-dated from section to section. Perfect cross-dating among the four cores in each of the many trees sampled in this manner practically eliminates the possibility of incomplete layers at the level where the borings were made. Completeness of growth rings is certainly the very general rule for trees on good soils in this region.

DISCUSSION AND CONCLUSIONS

It has been generally assumed, and quite naturally, that the nature of the ecotone and the present position of northern tree limit are determined by atmospheric factors (in common usage, by climate) acting

⁴ Since variations up to 0.1 mm. may occur in measurement, any difference of less than that amount is not significant.



FIG. 27. Small, angular rock fragments weathered from bedrock illustrate early stages in the formation of a residual soil. Island in Richmond Gulf.

directly on the trees. This implies that across the ecotone there is a climatic gradient in which the resultant of atmospheric factors becomes progressively less favorable for tree distribution in the north portion of the ecotone and a prohibitive influence at the present tree limit. That a climatic gradient does exist is undoubtedly true, but in the light of data here presented, it is obvious that climate is neither the local limiting factor in the Richmond Gulf portion of the ecotone nor the factor preventing trees from growing north of tree limit in this region.

Current knowledge of both the climate of this region and of the climatic tolerance of the species involved is not adequate for proving the above assertions. The trees themselves, however, give evidence in their growth-rings more satisfactory than many columns of climatic data.

FACTORS CONTROLLING LOCAL DISTRIBUTION OF TREES

The fact that trees occupy practically all habitats where soil occurs regardless of exposure, being absent only from habitats of essentially bare rock, suggests that climate is suitable for trees and that the direct factor preventing the occurrence of forest over all land surfaces is lack of soil. As was described above, geologic processes during and following the glacial

periods removed all soil and even loose parent material from most of this region. The period of sub-aerial history has been so short and the climate during that period has been so adverse to soil forming processes that very little soil has developed. Given sufficient time, with climate held constant, forest will develop over the whole area as soils of organic and inorganic nature develop and preliminary stages of succession occur. There is ample evidence that both sets of processes are in actual operation. In many dry situations small fragments weathered from bedrock already form a thin covering (Fig. 27). In moist places a layer of organic soil has come into existence and is spreading slowly over adjacent smooth rock surfaces (Fig. 28). Trees are taking root here, joining those already established in crevices (Fig. 29). More vivid evidence of succession from tundra to forest was supplied by study of duplicated photographs. In 1939, the writer succeeded in repeating three photographs made at Richmond Gulf in 1899 by the Canadian explorer and geologist, A. P. Low. The resulting pairs of photographs show that successional progress, though slow, is taking place at a rate sufficient to make itself evident after a lapse of less than half a century.

Conclusions drawn from growth-ring analysis strengthen those formulated from field observations.



FIG. 28. The spread of vegetation and development of organic soil over a rock surface kept moist by the melting of a large snow bank higher up the slope. Also, the rock surface illustrates polishing and grooving by glacial action. Mainland south of west tip of Cairn Island.



FIG. 29. Young trees take root in small rock crevices and hasten the spread of forest over areas now occupied by tundra. The taller trees average about 25 feet tall. Southeast slope of Cairn Island.

In the preceding section it was demonstrated that the collections used give an accurate representation of average tree growth in the Richmond Gulf region. A connection between atmospheric factors and certain described characters of growth-layers was suggested. For narrow layers it was possible to go further and demonstrate a positive relation to climate by showing that they cross-date over a wide area. And further, the conclusion that growth-ring analysis proves that this part of the ecotone has a forest climate was presented. This conclusion rests on the fact that trees on good soil have relatively large average radial growth, few relatively narrow rings, no critically narrow layers and no incomplete rings.

These conclusions demonstrate that, considered as a whole, the vegetation in this region has not achieved the level of climatic climax vegetation. The present patchwork of tundra and forest is a complex of successional stages all of which are pointing toward the development of the Subarctic Conifer Forest Plant Formation which is represented here by a plant association dominated by black and white spruces. The forest patches are approaching the climax level. The areas of tundra are edaphic subclimates which have far to go before reaching the relatively stable level of climax vegetation.

FACTORS CONTROLLING CURRENT NORTHERN TREE LIMIT

The conclusion that this region has a forest climate leads logically to the question as to whether this climate suitable for trees extends north of the current northern tree limit at Richmond Gulf. This question can be answered satisfactorily by determining how close the climate at Richmond Gulf is to the minimum for tree growth.

Several lines of evidence from field observations lead to the conclusion that the regional climate around Richmond Gulf presents conditions definitely above the minimum for the growth of trees. The types of evidence are as follows:

1. The forest is actively spreading from its existing footholds.
2. Trees on favorable soils are large, thrifty, and free from injury by atmospheric factors.
3. Near the margin of the range of a species, it frequently happens that individuals migrate outward into areas where climate is near the minimum for its existence. These individuals live for a number of years but are eventually killed off by a season of extraordinarily severe weather. The presence of several stands of trees containing individuals over 200 years old, and paucity of trees that have been killed or even injured by atmospheric factors indicate that nothing like this has happened around Richmond Gulf during the last two centuries.
4. Similarly, individual plants may grow vegetatively beyond the climatic boundaries in which they can reproduce. Many of the trees around Richmond Gulf bore an abundance of cones in

the summer of 1939. The abundance of seedling trees of all ages on burned areas proves that many of the seeds produced are viable.

5. Injury of trees by winds is limited to very local situations.
6. Areas where fires have destroyed forest stands are being reforested naturally. In one instance, study of an area photographed by Mr. A. P. Low in 1899 revealed that a fire had destroyed part of a stand between 1899 and 1914 and that in 1939 25-year-old spruce trees were already established in the burn.

At Beach Creek, a fire of unknown antiquity had destroyed most or all of the forest on the elevated delta. In 1939, trees had formed a dense forest at the margin of the delta. The presence of trees of all ages along the margin of the forest and scattered individuals all over the delta demonstrated that forest is actively spreading and will eventually cover the entire delta.

These two examples of the capacity of spruce forest to reestablish itself after a fire indicate a vigor which is not present in trees at a timberline controlled by climate.

Growth-ring analysis supplies a more quantitative measurement of climate because the degree of adversity of atmospheric factors is indicated by the character of the growth-rings of trees growing on good soil. As the atmospheric factors become progressively more unfavorable, the tree reacts first by narrowing of layers, later by incompleteness of layers and finally by missing rings (years in which no radial growth occurred).

The relatively great width of growth-rings in white spruce demonstrates beyond question that average climatic conditions are well above the minimum for the existence of that species. Preliminary analysis of black spruce and larch indicates that the same is true for those species.

As has been suggested, however, extreme fluctuations in climatic conditions are more important than average conditions. Absence of adverse extremes during the growing season is demonstrated by absence of "multiple growth-rings," that is, by the annual character of the layers. Infrequency of adverse extremes affecting an entire growing season is evident in the paucity of narrow rings. There are very few distinctly narrow layers and most of those present are not so narrow as to be especially conspicuous. For example, annual radial growth has been so uniform at Gulf Hazard during the last 230 years that only 41 growth-rings are sufficiently below average width to permit visual detection of the deviation; only 23 of these 41 layers are conspicuously narrow. At Great Whale River only 27 detectably narrow and only 11 conspicuously narrow layers have been formed in 160 years. Although the occasional narrow rings indicate lower than average conditions for those particular years, the fact that every tree studied had

formed a complete layer each year of its life proves that conditions have remained consistently well above the absolute minimum for the trees.

Evidence from growth-ring analysis thus supports the conclusion that tree growth in this portion of the ecotone has not been critically limited by climate during the last 250 years. Since major variations in climate take place very slowly, being measured in hundreds or thousands of years, it is logical to assume that climate has been favorable for trees for a much longer time than the observed period.

These conclusions concerning the degree of favorableness of atmospheric factors for tree growth around Richmond Gulf shed light on the question posed above as to what factor is determining the present location of northern tree limit. In the peripheral zone of the climatic range of species, growing conditions regulated by atmospheric factors are, of course, near the minimum for the existence of that species. The Richmond Gulf region is in the peripheral zone of the present range of black and white spruces and larch. The evidence here presented proves that climatic conditions in the region of the Gulf are well above the minimum for the growth of these trees. Assuming that the climatic gradient is a gradual one, the absence of trees along the coast immediately north of Richmond Gulf is not the result of a climate that is too severe for trees.

It would be very helpful to know what the soil conditions are beyond tree limit. Unfortunately the only recorded field observations in that region are the brief notes of A. P. Low, from which little of value concerning the ecology of the area can be gleaned. Since in going northward from Richmond Gulf the Labrador center of glaciation is left behind,

conceivably the amount of glacial soil materials may actually increase in that direction. Therefore it may well be that soil conditions are even less a limiting factor than around Richmond Gulf. But the only source of trees for migration is in the south, for all preglacial or interglacial forests, if any existed in the north, were destroyed by the ice flood. The northward spread of forest from far southern sources, limited as to available time and hampered by scarcity of soil, has not kept pace with post-glacial climatic amelioration. On the east coast of Hudson Bay, the potential climatic limit of forest under existing conditions is an unknown distance north of the actual limit at the present time.

SUMMARY

1. On the East Coast of Hudson Bay between Latitudes 53 and 56° North forest gives way to tundra across a broad transition zone or ecotone. This ecotone consists of a patchwork of communities of the Subarctic Forest Plant Formation and the Arctic Tundra Plant Formation.

2. This paper deals with the ecological character and environmental relations of the ecotone with special reference to factors restricting the occurrence of trees within the ecotone and factors determining the present position of northern tree limit.

3. The entire breadth of the ecotone was observed from the air. Intensive studies were conducted on the ground at Great Whale River and Richmond Gulf. The latter lies at the north limit of trees and the former is 80 miles farther south. Detailed analysis of tree growth-rings is used to support conclusions drawn from field observations.

4. Field observations revealed that trees grow on all areas of suitable soil (here used in the broad sense) regardless of exposure to atmospheric factors. Areas unsuitable for trees because of absence of soil are occupied by tundra. Trees are invading tundra areas as soil develops.

5. Growth-ring analysis proves that tree growth on good soils has not been critically limited in the past 250 years.

6. It is concluded that this portion of the ecotone has a forest climate. Occurrence of trees is limited by dearth of soil and not by atmospheric factors acting directly on the trees. The present regional vegetation has not achieved the level of climatic climax vegetation. The current patchwork of tundra and forest is a complex of successional stages tending toward the development of the Subarctic Forest Plant Formation represented here by a plant association dominated by black and white spruces. The forest patches are approaching the climatic climax level. The areas of tundra are edaphic subclimax communities that have far to go before reaching the level of climax vegetation. However, given sufficient time, with climate held constant, forest will occupy the entire area as soils of organic and inorganic nature

TABLE 2. Average layer width from cross sections and cores.

Tree number	Age	Average ring width
GWR174.....	88	1.64 mm.
173.....	97	1.28
165*.....	107	1.27
175.....	111	1.23
159.....	145	1.25
167.....	146	1.14
160.....	161	.95
157.....	170	.94
169.....	176	1.17
168.....	178	1.06
162.....	179	.79
GHI46B.....	90	1.53
146A.....	91	1.72
154.....	134	1.16
151.....	136	1.62
152*.....	142	.94
150.....	145	1.08
148.....	199	.40
149.....	214	.69
WR129*.....	173	.88

*Indicates measurements on cross section; the others were made on cores.

develop and preliminary stages of succession occur. Ample evidence that these successional tendencies are currently active is presented.

7. Dearth of soil is a result of the geologic history of the region. Continental ice sheets of the Pleistocene epoch removed all soil and even loose material from the bedrock and left very little debris behind when they melted. Most of what may have been left has been removed by stream wash, and also by wave action which progressively affected much of the region during the relative lowering of sea level in post-glacial time. The period of subaerial history has been so short and the character of the climate has been so adverse to soil forming processes that very little soil has been formed. Thus climate has had an indirect effect on the development of the current distribution of trees although it is not the direct factor restricting their occurrence.

8. The question, what factor is controlling the current position of northern tree limit is posed. Evidence is given that, contrary to the natural assumption, climate is not the direct factor preventing the northward spread of trees beyond the present tree limit. At the edge of the climatic range of a given species, atmospheric factors are at a minimum for individuals of that species. Growth-ring analysis and reproductive activities of the trees indicate that the climate at Richmond Gulf is not only possible for trees but is

actually somewhat above the minimum for their existence. Richmond Gulf is at the present north limit of trees. It is logical to assume that under the geographic conditions of this region, the climatic gradient is gradual not abrupt. Obviously, then, climate is not the factor determining the current north limit of trees. The potential climatic limit of forest under existing climate is an unknown distance north of the actual limit at the present time.

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